EDITORIAL REVIEW

THE ROLE AND EFFICIENCY OF ANIMALS IN UTILIZING FEED TO PRODUCE HUMAN FOOD

L. A. MAYNARD
Cornell University, Ithaca, New York

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In the face of famine situations throughout the world, measures have been taken in this country to curtail livestock feeding operations in the interests of conserving grains for shipment abroad. It has long been recognized that the production of meat, eggs, and milk from crops which might be consumed direct by man involves a large wastage of the basic food supply as measured in calories. For a complete diet, however, account must also be taken of protein, minerals and vitamins. Animal products are of special value as concentrated and palatable sources of these nutrients. What is their relative nutritional value? How do different animal species compare in efficiency in producing human food? To what extent can foods of plant origin satisfactorily replace milk, meat or eggs in terms of overall nutrition? Though the practical interest in these questions is, doubtless, a temporary one in this country, they will continue to be of basic importance in planning for better nutrition in many countries of the world. Thus, a discussion of them is here presented.

EARLIER STUDIES

In the midst of World War I Armsby ('17), in an article entitled "The Cost of Roast Pig," cited figures on the relative efficiency of the various classes of farm animals in converting

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grain into animal products for human food. Later, he ('20) discussed in detail the place of a livestock industry in general, and of various classes of animals in particular, with reference to the most effective utilization of our food resources. His opening sentence indicates a situation paralleling the one we face today. It reads: "The experiences of the great war have forced us to realize as never before that the maintenance of the food supply is the basal problem of civilization." He called attention to the large losses which occur in the conversion of wheat into meat as compared with its direct consumption.

Pearl ('20) classified foods derived from vegetable crops and aquatic animals as primary, and those derived from domestic animals as secondary, making the distinction that the secondary products involve large losses of potential food in their production. His data showed that from 1911 to 1918 the secondary foods furnished 39% of the total calories consumed, indicating that the nation's nutrition was on a wasteful economic basis. Earlier, Armsby, as reviewed by Forbes ('26) had stressed the same point in computing that farm animals consumed 3.9 times as much food on an energy basis as people, and that the grain they ate represented 2.5 times that required to feed the human population. The latter estimate is borne out by a recent calculation by Harper and Hyer ('43) who further pointed out that Illinois, Iowa and Nebraska alone produce enough corn to meet our calorie needs, yet all but 6% is fed to animals.

The literature contains many reports of feeding trials dealing with the efficiency of farm animals in utilizing feed to produce human food. Data from the earlier reports have been summarized in the monograph by Armsby and Moulton ('25). Later publications which contain pertinent data for the various species are those of Hogan, Weaver, Edinger and Trowbridge ('25); Mitchell et al. ('28); Mitchell and Hamilton ('29); Mitchell, Card and Hamilton ('31); Forbes and Voris ('32); Maw ('33); Edwards ('36); and Hershaw ('38). An especially noteworthy recent publication is the one by Leitch

and Godden ('41), which sets forth data on the relative efficiency of various farm animals as calculated from the intakes in rations recommended by the Ministry of Agriculture of Great Britain for selected levels of production and feeding situations.

The data in these various publications are highly variable for a given species. This is not surprising when one notes the diverse bases on which the calculations are made, and the varying conditions in the feeding trials in question. The feed intake and product are sometimes expressed in pounds and sometimes in gross, digestible, or net calories. In some cases, inedible products are included in the calculations of the energy representing human food produced. Other variable factors which markedly affect the results are: level of production, balance of ration, level of feeding in relation to productive capacity, and length of feeding period under consideration with respect to the normal production cycle for the species in question. Unsound comparisons have been made in some cases because of a failure to appreciate the significance of these different factors. With these variables in mind, the writer has reviewed the various publications reporting feeding trial data, with the objective of getting some useful answers to the questions listed in the first paragraph of this article.

THE EFFICIENCY OF ANIMALS IN ENERGY CONVERSION

The figures shown in table 1 have been arrived at as representing roughly the efficiencies of animals in converting gross energy of their feed into gross energy of edible products. The limitations of gross energy as a measure of feed value are obvious, but no other single measure is applicable to all the species concerned. The digestible energy of a feed as measured with cattle or sheep clearly is not applicable to poultry, or even to hogs. Net energy values are obviously even less suitable. The figures in the table are based on somewhat better than average levels of production, obtained under good feeding practices. The data for the dairy cow are based on

the lactation cycle including a dry period, involving a year's production of 7,000 to 8,000 pounds of milk. Those for the hog cover the period from weaning to slaughter at approximately 230 pounds weight. The data on beef cattle and sheep are limited to feed-lot studies because of the impossibility of measuring the feed intake from pasture or range. The limitations of these data for comparative purposes are recognized. The beef cattle studies in question were made with animals starting around 450 pounds and carried to from 850 to 1,200 pounds in the different experiments considered. The figures for lambs were obtained with animals fed from a

TABLE 1

The recovery in edible products of gross calories of feeds when fed to livestock.

	PERCENTAGE RECOVERY OF GROSS CALORIES IN EDIBLE PRODUCT	
	Estimated range	Estimated single figure as average
Pork	15–25	20.0
Milk (dairy cows)	12–18	15.0
Eggs	6– 9	7.0
Poultry meat	4– 6	5.0
Beef	3– 6	4.0
Lamb	3– 6	4.0

weight of approximately 65 pounds up to a market weight of around 90 pounds, giving no credit for the wool produced. The data for the production of poultry meat cover the period from hatching to market, and those for egg production represent a year's feeding period at a production level of about 150 eggs per year.

The range of values given in table 1 reflects the varying results obtained in different trials, explainable in part by differences in level of production or length of feeding period, or other variables necessarily present even under the narrow range of conditions governing the selection of the basic data. The data in the last column are considered to be the values which might be chosen to represent the data as a whole for a

given species. Of course any set of figures such as those presented in table 1 are subject to challenge because they involve a somewhat arbitrary selection of the data and feeding conditions upon which calculations must be based. It is believed, however, that they present a trustworthy picture of the order of efficiency of the different species as converters of gross feed energy into gross energy in the edible product, under the conditions specified. Clearly, pork production is most efficient, followed rather closely by milk production. The production of eggs rates much lower in efficiency than that of milk, but outranks the production of poultry meat, beef, or lamb.

A particularly important consideration, from the standpoint of the utilization of our potential food resources, is the extent to which the data obtained from feeding trials under the better than average conditions specified may be considered to apply to actual practice. Here, some recently published figures of Jennings ('43) are of special significance. These data, set forth in a condensed form in table 2, take into account the feed consumed and the animal products yielded based on average data for the country as a whole. The reader of this bulletin will appreciate that many assumptions are necessarily involved in the calculations made. The "feed unit" is arbitrarily chosen and the values assigned to certain feeds can be criticized, but so could any other single set of values which are chosen to apply to all feeding operations. Despite the limitations which are inherent in the various calculations involved, it is believed that the data in table 2 present very useful information on the order of efficiency of the production of human food by animals, as now occurring in the country as a whole.

The data for "all feed" indicate, on an entirely different basis, practically the same order of efficiency as shown in table 1. The only marked exception is that the production of poultry meat is given a considerably higher relative rating in Jennings' table. The fact that similar orders of efficiency are revealed from the two widely different bases of calcula-

tion lends confidence to the interpretations which are made as to their signficance. The different bases must be kept in mind, however, in any discussion of these data. The data in table 1 indicate what might be expected to result with good feeding practice, while those in table 2 reveal what we are doing at present on a nation-wide basis.

From the standpoint of competition with man's food supply, a comparison based on the entire feed consumption, particularly when expressed as gross energy, is not a fair one because sheep and cattle consume large amounts of roughage, pasture grass, and fibrous by-products inedible by man. Here, they actually increase the human food supply in converting

TABLE 2

Feed units required to produce 2600 Cal. of human food.

ANIMAL PRODUCT	ALL FEED	CONCENTRATES
	lbs.	*lbs.*
Pork	7.7	7.2
Milk (dairy cows)	9.3	2.3
Eggs	21.9	20.6
Poultry meat	29.9	27.1
Beef	71.6	15.2
Lamb	74.5	4.7

[1] Adapted from table 36 in a publication by Jennings ('43).

these feeds into meat or milk. In this connection the data headed "concentrates" in table 2 are pertinent. The data indicate that, per unit of feed consumed which might, for the most part, be eaten directly by man, the greatest return can be obtained in milk. Pork production drops below lamb production and the poultry industry becomes the least efficient. Of course, this comparison is faulty, in considering the over-all utilization of potential food resources, to the extent that the pasture grass or hay is frequently produced on land that might be effectively used for the production of crops to be consumed directly.

Further, one must avoid over-emphasis of the significance of these data as a whole. There is much more variation in

the amount of concentrates fed for a given feeding operation in different situations than is the case for total feed. For example, under certain conditions substantial amounts of milk are being produced from roughage alone, while in intensive dairy areas, particularly where pasture is limited, the rate of concentrate feeding is markedly higher than indicated by the average data in table 2. It should also be noted that where a few hogs or chickens are kept on the farm and consume products which otherwise would be wasted such as cull fruits, vegetables and seeds, kitchen wastes, etc., a contribution to the human food supply is made similar to that of cattle and sheep eating roughage. On the other hand, commercial egg production represents the most critical competition for man's food supply of any feeding operation, in that it calls for the feeding of substantial amounts of special vitamin sources such as cod liver oil and riboflavin products needed as supplements to certain human diets.

EFFICIENCY OF ANIMALS IN PRODUCING PROTEIN

Energy conversion is the best single measure of the efficiency of utilization of the feed supply as a whole, but nutritionally there are other considerations which are more important in determining the amounts and kinds of animal products which should be produced. If energy were the sole consideration, there could be no nutritional justification for animal products at all, except milk for the very young, because energy needs can be met from vegetable sources. Animal products have a special value in the diet as rich sources of protein which in general are superior in nutritional quality to vegetable sources. In table 3, data are presented to show the relative efficiency of protein production by various species. These data are taken from feeding trials similar to those considered in setting up table 1. The protein intakes were judged adequate in amount and kind, but not excessive. The first column of figures reveals the order of efficiency of edible protein production in terms of the total ration measured as gross energy. The second column indicates the order of

efficiency in converting feed protein into that for human food. It is noted that on both bases milk production leads, with beef and lamb production showing the lowest efficiency.

TABLE 3

The efficiency of protein production by various animal species.

ANIMAL PRODUCT	EDIBLE PROTEIN PRODUCED PER THERM [1] OF DIET	EFFICIENCY OF CONVERTING FEED PROTEIN INTO EDIBLE PROTEIN
	gm	%
Milk (dairy cows)	4.0	14.5
Eggs	5.4	9.5
Poultry meat	5.1	13.4
Pork	4.0	14.5
Beef	2.0	8.4
Lamb	1.7	4.4

[1] 1,000 Cal.

ANIMAL PRODUCTS AS SOURCES OF MINERALS AND VITAMINS

Animal products are also of special value in the diet as sources of minerals and vitamins. While the body needs at least thirteen different mineral elements in its food, and probably as many different vitamins, the practical importance of these nutrients is limited to the cases of those which are likely to be lacking in the diet unless attention is given to them in food selection. A list of the minerals and vitamins that are of practical importance, insofar as any of the animal products under consideration is a significant source, is presented in table 5. Since the first essential is to provide a diet with sufficient calories, and since the previous discussion has dealt with the efficiency of calorie production, the mineral and vitamin data are presented as the amounts furnished by a therm (1,000 Cal.) of a given product. This number of Calories represents one-third of the recommended allowance for a moderately active man. Of course, no single animal product is consumed to the extent of one-third of the diet, but the basis selected is useful for comparative purposes.

Since calcium cannot be supplied adequately by any combination of vegetable foods which our people could be expected to eat, its high content in milk makes this food of special importance. A quart of milk a day supplies the full recommended allowance (Food and Nutrition Board, '45) for a growing child except during adolescence, and more than is needed for the adult, except for expectant and nursing mothers. It would take forty-five eggs to supply as much. Other animal products are negligible contributors of calcium. While phosphorus is needed in large amounts by the body, and while animal products are all good sources, this mineral is not considered here because it commonly presents no dietary problem.

The iron needs of the body can be met adequately from vegetable sources alone if the foods are properly selected, but certain animal products represent the most convenient way of obtaining an adequate supply, particularly when the needs are largest. It is noted in the table that eggs far surpass other animal products as a source of this mineral. Beef, poultry meat and lamb supply approximately one-half as much, and pork and milk less than a quarter.

As a source of vitamin A, eggs are markedly superior to its nearest competitor, milk, on a caloric basis. The other animal products are negligible sources. While eggs and milk are important dietary constituents from the standpoint of vitamin A, it is possible to meet body needs from vegetable sources alone.

Nearly half of the thiamine consumed in the current U.S. diet is supplied by animal products. Among these products pork and eggs greatly outrank the others, as is shown in table 4. To meet our thiamine needs without animal products would call for an increased consumption of whole grain or enriched cereals, or a higher level of enrichment.

Riboflavin is very difficult to get adequately from vegetable foods. Milk is the best source, followed by eggs. A quart of milk will furnish 60% of the adult daily allowance. It would require ten eggs to furnish as much. On a calorie basis,

the values for different meat sources range from one-third down to one-eighth of the figure for milk. A decreased supply of riboflavin from animal sources would call for an increased consumption of leafy vegetables, pulses and nuts.

The extent of the need for niacin and its distribution in foods are less well known because the knowledge regarding this vitamin is so recent. Meat products, notably poultry meat, have a special value as a source of this vitamin, in contrast to milk and eggs which are unimportant sources. Whole wheat products are excellent sources, but refined are not, unless enriched. Pulses and nuts also supply liberal amounts.

TABLE 4

Minerals, vitamins and protein supplied by animal products per therm of edible material.

ANIMAL PRODUCT	CA	FE	VIT. A	THIAMINE	RIBOFLAVIN	NIACIN	VIT. D	PROTEIN
	mg	*mg*	*I.U.*	*mg*	*mg*	*mg*	*I.U.*	*gm*
Pork	15	4	0[1]	1.6	0.3	7.1	0	26
Milk (dairy cow)	1710	3	2319	0.6	2.5	1.4	38	51
Eggs	342	17	7215	0.8	2.2	0.6	600	81
Poultry meat	83	10	0	0.6	0.9	44.3	0	104
Beef	37	10	0	0.4	0.5	17.5	0	65
Lamb	29	8	0	0.6	0.7	16.6	0	50

[1]A zero indicates none or negligible amounts.

Vitamin D is not adequately supplied by any natural food, and vegetable products in general contain none. Where a lack of sunshine makes dietary vitamin D essential, special sources such as cod liver oil or vitamin D milk must be resorted to. Among the animal products, except the special milk mentioned, eggs are the only significant contributor.

The data in table 4 show that on an energy basis, poultry meat supplies more protein than other animal products. Eggs excel beef, milk and lamb; pork ranks low because of the relatively large amount of fat contained in this product. The efficiency of protein production has been considered earlier. It is well recognized that animal protein has a higher nutritional efficiency than most vegetable proteins, but whether

a certain minimum intake of animal protein is essential for optimum nutrition remains an open question.

Any overall nutritional rating of animal products is necessarily subject to the limitation, as indicated in table 4, that no one is superior to another in all nutrients. It seems clear that milk should be given first place, particularly in view of its special value and suitability in the diet of the young. In fact, only this last consideration justifies ranking milk above eggs which actually surpass milk in some of the nutrients under consideration. Pork would appear to deserve a somewhat higher rating than the other meats because of its superiority in the critical vitamins, thiamine and riboflavin.

TABLE 5

The minerals, vitamins, and protein supplied by certain animal products per therm of gross energy consumed.

ANIMAL PRODUCT	ONE THERM OF GROSS ENERGY WILL PRODUCE							
	Ca	Fe	Vit. A	Thiamine	Riboflavin	Niacin	Vit. D	Protein
	mg	*mg*	*I.U.*	*mg*	*mg*	*mg*	*I.U.*	*gm*
Pork	3.0	0.8	0 [1]	0.30	0.10	1.4	0	5.2
Milk (dairy cows)	256.0	0.9	348	0.09	0.38	0.2	5.7	5.7
Eggs	24.0	1.2	505	0.06	0.15	0.04	42.0	5.7
Poultry meat	4.2	0.5	0	0.03	0.05	2.2	0	5.2
Beef	1.5	0.4	0	0.02	0.02	0.7	0	2.6
Lamb	1.2	0.3	0	0.02	0.03	0.7	0	2.0

[1] As in table 4.

A combined picture of the data in tables 1 and 4 are presented in table 5. Here it is revealed that despite its superiority as a converter of calories in terms of all feed consumed, the hog ranks below the cow and the laying hen as a producer of most of the nutrients which make animal products of special value in the diet. Pork production does, however, excel the other meat-producing operations with respect to most all of the nutrients under consideration.

In considering the competition of animals with man for the basic food supply, it is worth noting that cattle and sheep require no thiamine, riboflavin or niacin in their feed because

these vitamins are synthesized by rumen action. On the other hand, pigs and chickens do require a ration containing these nutrients even as does man. The practical importance of this competition is illustrated by the case of riboflavin. Large amounts and special sources of this vitamin, which is difficult to provide adequately in the diet of man, are required for growth and egg production in poultry. Eggs are nearly as rich in riboflavin as is milk, but the vitamin in the latter can be furnished by the cow herself, whereas that in the egg represents only a small percentage recovery of the riboflavin in the feed.

ORDER OF NUTRITIONAL IMPORTANCE OF LIVESTOCK OPERATIONS

It is clear from the data presented that any proposed adjustment in the production of animal products as a means of conserving the basic food supply should be considered on a differential basis, having in mind both economic and nutrition aspects. On the basis of both efficiency of production of nutrients and of overall nutritive value of the product, milk production should have first priority on the feed supplies available.

An effort should be made to use pasture, hay and silage to the fullest possible extent in feeding dairy cows so as to decrease the need for concentrates which can be used direct by man. At the same time, it must be recognized that the efficiency of milk production increases with the addition of concentrates to a ration of roughage only. Enough concentrates must be allotted to dairy cows to take full advantage of their productive efficiency.

It should be stressed that what has been said about milk production does not apply to butter unless the skimmilk is fully utilized as human food — which is the case to a very limited extent. While butter is a highly prized food, it represents, nutritionally, the less important part of milk. All of the protein, minerals, and most of the vitamins, are in the by-product. According to the consensus of present scientific

information, there is no nutritional disadvantage in substituting vegetable fats for butter in the human mixed diet if the vegetable fat is appropriately fortified with vitamin A. Clearly a real effort should be made to recover, for human use, as much as possible of the skimmilk now wasted from butter manufacture. While much of it is used to feed chickens and hogs, this is, nutritionally, a very inefficient use compared to direct consumption.

Nutritionally, egg production ranks close to milk production, but in terms of its competition for man's food supply, it must be rated markedly lower in priority in considering changes which call for a decrease in the production of animal products. The hen should, however, be rated above the hog, the other chief competitor for man's food, as indicated by the data previously presented and discussed. Certainly, considering the poultry enterprise as a whole, the feeds available should be used primarily for egg production rather than for poultry meat. The latter would appear to have the lowest rating of any of the meat-producing operations.

To the extent that beef cattle and sheep can utilize range or other roughage not available to dairy cattle, their production should have a high priority rating, even though they are inefficient producers of nutrients. When large amounts of concentrates are involved in the feeding operations, however, the situation is very different. As far as possible, beef cattle and lambs should be fed grains only to the extent that fattening is necessary to produce meat of acceptable quality. They should be slaughtered at the lighter weights.

The carcass increase that results during the heavy fattening is less valuable for the nutrition of man than that produced earlier because it contains an increased proportion of fat which possesses energy value only. This fact is illustrated clearly by the results of studies with pigs by Hogan, Weaver, Edinger and Trowbridge ('25). These data show that, as the pig grows, more feed energy is needed to produce a pound of protein and that at each additional weight, relatively more fat and less protein tissue is formed. The highly

fat carcass also contains relatively less of the minerals and vitamins which make meat of special value in the diet. There is no nutritional justification for feeding meat animals to produce fat, for vegetable fats will serve as well in the diet. Further, much of this fat of highly fattened animals is wasted insofar as human consumption is concerned. This is particularly true in the case of sheep and cattle.

OTHER FACTORS TO BE CONSIDERED IN EVALUATING THE LIVESTOCK INDUSTRY

The data here presented relate solely to the efficiency of feed utilization. Production per acre and per unit of labor also require consideration in rating the various livestock operations. Data summarized by Hill ('43) indicate that milk should be ranked first on these counts also, considering the production of nutrients of special importance to man.

It is recognized that in the foregoing discussions of animal efficiency the animals have not been given credit for non-food products. Some of the feed wastage is recovered in manure, which indirectly is an important contribution to the food supply through the maintenance of soil fertility. Lambs have been given no credit for the wool they produce. Slaughtered animals contribute hides, inedible by-products for animal feed, and are a source of other important special products. These secondary contributions must be taken into account in fully assessing the place of a livestock industry in the agricultural and industrial economy.

SUMMARY

Both animals and man draw on the same basic food supply — the products of the soil. While the feeding of animals results in a concentration of food nutrients into more palatable and more digestible forms for human use, it also involves a wastage of potential food supplies in the process. Any adjustment of livestock production in the interests of better nutrition for more people should be on a differential basis, recognizing differences among species and feeding operations

with respect to (a) efficiency of human food production, (b) consumption of foods directly useful to man, and (c) overall nutritive value of the animal product. Taking these factors into account, it is concluded that:

1. Market milk production should have first priority on feed supplies available. Butter production rates the same priority only to the extent that the skimmilk is recovered for human use.

2. Egg production should be ranked above pork production, but broiler production deserves only a very low rating.

3. To the extent that beef cattle and sheep utilize range and other roughage not available to dairy cattle, their production should have a high priority rating, but this is not true where any large consumption of concentrates is involved. The heavy fattening of meat animals is a very inefficient use of the basic food supply in relation to human needs.

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THE ADEQUACY OF INTRAVENOUS PARTIAL ACID HYDROLYSATES OF CASEIN AND FIBRIN FOR NITROGEN BALANCE IN DOGS

DOUGLAS V. FROST AND WILLIAM C. RISSER
Abbott Laboratories, North Chicago, Illinois

THREE FIGURES

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In intravenous studies with dogs (Risser, Schenck and Frost, '46a, '46b), partial acid hydrolysates of casein fortified with cysteine hydrochloride monohydrate at about 2.8% of the dried hydrolysate gave nitrogen balance at about the same minimum levels of nitrogen as were needed for complete acid hydrolysates of casein fortified with both cysteine and tryptophane, i.e., about 140 mg nitrogen per kilo per day for each. This appeared as presumptive evidence that the peptide fraction of partial acid hydrolysates of casein is well utilized on injection. In further studies with hypoproteinemic dogs (Frost, Heinsen and Olsen, '46), wherein high levels of nitrogen were given intravenously, i.e., 600 mg N per kilo per day, fibrin hydrolysate was utilized far more completely than casein hydrolysate fortified with either cystine or cysteine hydrochloride monohydrate at levels of about 2% of the dried hydrolysate. A comparison of the essential amino acid content of the two hydrolysates was reported in the latter paper.

The object of these experiments was to maintain dogs over a period of many weeks on minimum levels of the partial acid hydrolysates of casein and fibrin. Casein hydrolysate was used "as is" and with an addition of cystine to give a level only slightly below that present in fibrin hydrolysate. Since casein

hydrolysate was shown by analysis to contain more methionine than fibrin hydrolysate, this experiment would show that any superiority of fibrin hydrolysate over casein hydrolysate was not due to a difference in sulfur amino acid content, and must, therefore, be explained on another basis.

The long-term intravenous use of fibrin hydrolysate at a low level of nitrogen input provided data which appeared suitable for application of the graphic method of Allison and Anderson ('45) for estimation of the nitrogen balance index of the preparation.

METHODS

Adult female dogs were placed on a highly purified non-protein diet as follows: 73 gm sucrose, 20 gm lard, 3 gm corn oil, 0.5 gm Haliver Oil, 4 gm U.S.P. salt mixture I, 0.1 gm choline chloride, 1 gm agar, 0.6 mg thiamine hydrochloride, 0.6 mg riboflavin, 12 mg nicotinamide, 0.4 mg pyridoxine hydrochloride, and 1.2 mg calcium pantothenate. The diet supplied 5.1 cal. per gm. To avoid possible vitamin deficiencies during periods of decreased voluntary food intake, biweekly injections of a specially prepared B-complex solution were made to supply per week per kilo body weight: thiamine 0.5 mg, riboflavin 0.5 mg, nicotinamide 15 mg, pyridoxine 0.3 mg, and calcium pantothenate 1 mg. Percomorph liver oil was given by drop during the long injection periods at a rate of 2000 units of vitamin A and 200 units of vitamin D per week. A concentrate of vitamin B_c [1] was given orally equivalent to 10 gm of 70% alcohol-insoluble liver extract per week.

Food consumption was recorded daily. An amount of the non-protein diet calculated to supply 80 cal. per kilo per day was offered and any portion not consumed was accounted for. Preliminary to beginning an experiment the dogs were placed 2 to 4 weeks on the above diet supplemented with dried fibrin in an amount equal to 100 mg N per kilo body weight per day, a level previously shown to be adequate for nitrogen balance.

[1] Supplied by Dr. F. C. McIntire.

The standard weight of each dog was taken as the kennel weight at the time the experiment was begun and calculations for nitrogen intake were made on the basis of this standard weight. Since the dogs were fed a highly nutritious and varied diet in the kennel, they were generally at maximum weight at the time they were shifted to the basal diet.

For injection, the dogs were placed on a convenient body support with their legs hanging in natural downward position and held lightly by leather straps. Injections were made into the cephalic and saphenous leg veins in rotation, so that any single vein was used only once in 4 days. Injections were carried out in a regular 2-hour period each morning. The rate of injection ranged from about 0.7 to 1.3 mg nitrogen per kilo body weight per minute depending on the nitrogen input.

Seven-day periods for injection and collection of excreta were observed. The feces were labeled with carmine at the end of each period. The feces and urine for each period were thoroughly mixed in a Waring blendor for convenience in sampling. It was determined by preliminary experiment that this procedure gave total nitrogen excretion values comparable to the values obtained by separate analyses of the urine and feces. Nitrogen determinations were made by macro-Kjeldahl, using 10 ml samples.

The hydrolysates used were prepared from commercial casein and fibrin essentially according to the method of White and Elman ('42). These hydrolysates contain about 0.5 and 1% tryptophane, respectively, on a dry basis.[4] The degree of hydrolysis effected by the conditions of hydrolysis have been studied by Frost and Heinsen ('45). The particular preparations used herein represent 6-hour hydrolysis with 2.8 N sulfuric acid and contain about one-third of the amino acids in free form. The solutions used were sterile-filtered and were non-pyrogenic by U.S.P. test. All solutions contained 5% protein hydrolysate corresponding to about 0.7% nitrogen. Dextrose was added, where indicated, at a 5% level. Cystine was added at 0.08%, the maximum of solubility. This

represents an addition of about 1.6% cystine on the basis of the dry hydrolysate and a ratio of 1 gm of the amino acid to about 9 gm of total nitrogen. Since the casein hydrolysate on a dry basis contained about 0.6% cystine, the total cystine content was about 2.2%.

It is generally recognized that many factors influence the minimum level of protein intake needed to maintain nitrogen equilibrium. These factors have been well described by Melnick and Cowgill ('37). In the present study it was decided to approach a state of nitrogen equilibrium from the side of negative balance, as recommended by the above authors.

EXPERIMENTAL

Casein hydrolysate

Two dogs which had been kept many months on experiment at near maintenance levels of nitrogen were given casein hydrolysate. Dog 7 was started at a level of 105 mg N per kilo and dog 8 at 125 mg N per kilo. Both dogs lost weight rapidly. Weight loss continued even when the nitrogen intake was increased to 130 and 160 mg N per kilo, respectively. Dog 7 developed an infection and died at the end of the fifth week. Dog 8 declined continually and died after 9 weeks' injection.

Dogs 10 and 11 were given casein hydrolysate with added dextrose and cystine. The level of nitrogen was increased from 120 mg to 140 mg and finally to 160 mg N per kilo per day. Both dogs were in severe negative balance throughout and the experiment was discontinued in order to spare the dogs. Biopsy samples were obtained from the livers of dogs 10 and 11 after 5 and 3 weeks, respectively.

A further experiment was conducted with dog 12 in which casein hydrolysate was injected at 160 mg N per kilogram for a period of 4 weeks. A negative balance of 8, 4, 4, and 2 gm N was determined in each of the 4 weeks successively. Body weight fell from 5.35 kg to 5 kg in the same period.

Fibrin hydrolysate

Dogs 3 and 5, which had been maintained for more than a year on near maintenance levels of nitrogen, either oral or injected, were placed on fibrin hydrolysate at levels well below the previously established maintenance level of about 120 mg N per kilo per day. Both dogs were held at levels below 100 mg N per kilo for 5 weeks during which there was gradual loss of weight. The negative balance during this period was consistently small in both dogs. On increasing the input to 110 mg N per kilo, both dogs went into slight positive balance but weight loss continued. Food intake was constant but somewhat subnormal. After 8 weeks' injection, a preparation containing fibrin hydrolysate plus dextrose was introduced to see if this combination would stem the weight loss and improve nitrogen utilization. When no advantage was seen after 2 weeks, the level of nitrogen was increased to 120 mg per kilo per day. However, weight loss continued and after 2 weeks more both dogs were given 100 mg cystine daily by capsule. In the ensuing 2 or 3 weeks, the dogs showed a small weight response and apparent improvement in nitrogen utilization.

Dog 3 became severely ill with a respiratory infection and was sacrificed at the beginning of the fifteenth week of injection after 2 days' illness. The dog was autopsied to determine whether or not liver damage was apparent. A liver biopsy was performed on dog 5 at the end of the fifteenth week of injection to compare with the autopsy samples from dog 3.

RESULTS

Records of nitrogen and weight balance for four dogs which received the various casein hydrolysates are shown in figure 1. The record of dog 12 receiving casein hydrolysates unfortified is not shown, but was comparable to the above in that nitrogen and weight balance were not attained.

Weight and nitrogen balance records for dogs 3 and 5, which received fibrin hydrolysate over 14–15 weeks, are shown

in figure 2. The levels of nitrogen given per kilo body weight, shown in parenthesis, were calculated on the standard kennel weights of the two dogs, which were 7.16 kg for dog 5 and 6.6 kg for dog 3. Nitrogen input calculated on the changing weights of the dogs shows an increasingly higher input per

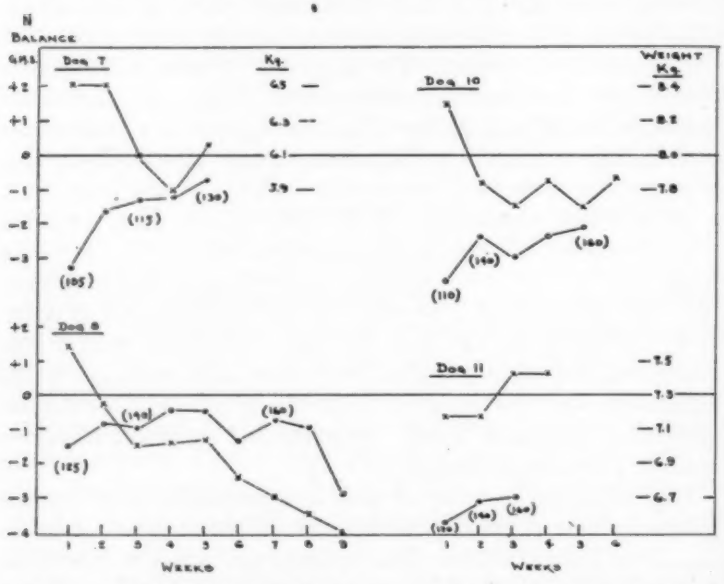

Fig. 1 Weight and nitrogen balance curves for dogs receiving acid hydrolysates of casein. Milligrams nitrogen injected per kilo per day are shown in brackets adjacent to the nitrogen balance curves. Dogs 7 and 8 received casein hydrolysate, 5% unfortified; both dogs died thus terminating the experiment. Dogs 10 and 11 received casein hydrolysate, 5%, with added dextrose, 5%, and cystine, 0.08%. The experiments were terminated to spare the dogs and to get liver biopsy samples.

kilo as the weights of the dogs declined. Thus at the lowest weights of the two dogs, i.e., 5.12 kg for dog 5 and 5.23 kg for dog 3, the input was 170 mg and 160 mg N per kilo, respectively, as against 120 mg N per kilo for both dogs based on their standard weights.

The voluntary food intake averaged about 50 cal. per kilo per day, based on the standard weights of the dogs. Where dextrose was given in conjunction with the hydrolysates, the caloric input in this form ranged from about 3–4.5 cal. per kilo from the smallest to the largest daily injection, i.e., levels

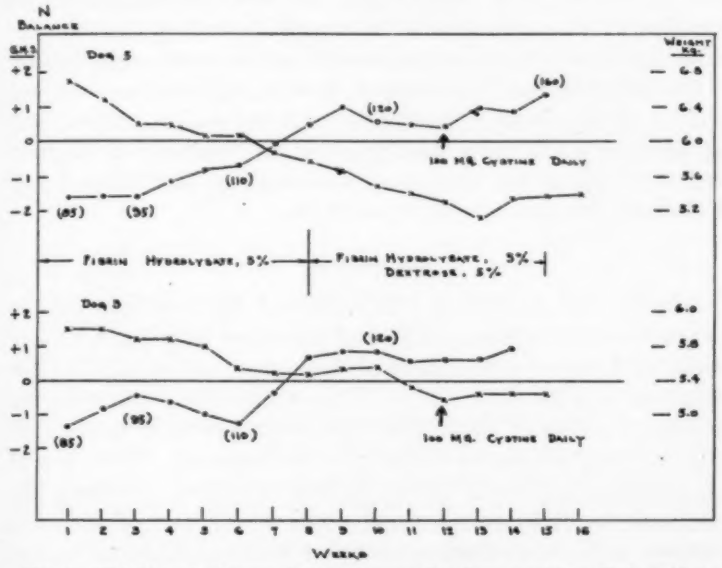

Fig. 2 Weight and nitrogen balance curves for dogs receiving fibrin acid hydrolysates. Milligrams nitrogen injected per kilo per day are shown adjacent to the nitrogen balance curves. Dog 3 was finally sacrificed following a 2-day respiratory infection and was autopsied. Dog 5 was taken off experiment at the same time in order to obtain a liver biopsy sample.

of 110–160 mg N per kilo per day. The added dextrose therefore, accounted for less than 10% of total caloric intake.

Dog 3, autopsied after 14 weeks on fibrin hydrolysate, showed normal kidney, heart, spleen, and adrenals.[2] The liver of this dog showed a strong, positive fat stain, fatty infiltration, marked cloudy swelling, and cellular vascularization.

[2] Dr. R. K. Richards performed the autopsy, the liver biopsies, and the microscopic examination of liver slices.

The liver sample obtained from dog 5 by biopsy also showed a strong positive fat stain and fatty infiltration. The cytoplasm was coursely granular and swollen and contained large vacuoles. The liver biopsy sample from dog 10 after 4 weeks on casein hydrolysate with cystine showed a weak, positive fat stain, fatty infiltration, and some granulation and swelling in the cytoplasm. The sample from dog 11 had negative fat stain, but some cytoplasmic swelling and granulation.

No difficulty was experienced in carrying out the daily injection of the fibrin hydrolysate over the entire period, and no damage to the veins was apparent. A thinning of the hair and scaliness of the skin became evident in both dogs 3 and 5 toward the end of the experiment.

DISCUSSION

Allison and Anderson ('45) derived the relationship between nitrogen balance (NB) and absorbed nitrogen (AN) to yield an indirect evaluation of biological value (BV). The equation is $NB = BV(AN) - EN$; where EN represents endogenous nitrogen excretion. The assumption is made that EN is a constant under ordinary circumstances. When a plot of nitrogen balance is made against absorbed nitrogen, the slope of the line represents the rate of change of nitrogen balance with respect to absorbed nitrogen. This value has been designated as the nitrogen balance index by Allison, Seeley, Brown and Anderson ('46) with reference to the classical concept of biological value as defined by Mitchell ('24, '44).

The present studies offered an opportunity to apply the above principle to derivation of a figure for the nitrogen balance index of fibrin hydrolysate given intravenously. A plot of the nitrogen balance data for dogs 3 and 5 is shown in figure 3. The changing weights of the dogs for each period were used in reducing the weekly values to the basis of milligrams per kilo body weight per dog. Although the points show considerable scatter, it is possible to construct representative lines for each dog. The slopes of the lines as

drawn, representing the nitrogen balance indices, are 0.83 and 0.86 for dogs 3 and 5, respectively. It will be noted that our results are, of necessity, expressed on a per kilo weight basis whereas those of Allison and Anderson ('45) are on a per square meter surface basis, calculated on the changing weights of the dogs.[3]

The limiting deficiency in fibrin hydrolysates at the low level of input used has not been determined. The fatty infiltration seen in the livers of dogs 3 and 5 is suggestive of

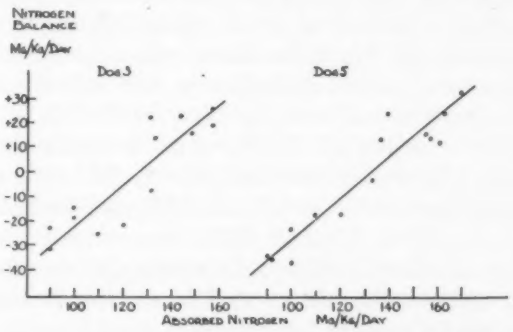

Fig. 3 A plot of nitrogen input vs. nitrogen balance for dogs 3 and 5. The curves are assumed to originate at 0 nitrogen input. The equation describing the curve is, $NB = k(AN) - NE_0$, where NB is nitrogen balance, AN is absorbed (or injected) nitrogen, NE_0 the excretion of nitrogen on a protein free diet (assumed to be a constant), and k the slope of the line. k is equal to that fraction of nitrogen input retained in the body, the biological value, or nitrogen balance index.

methionine deficiency as observed in other species. The slight response to cystine addition in these dogs may also be of significance. Madden et al. ('43) have stressed the importance of methionine for long-continued intravenous nutrition in dogs and have described the more dynamic effects of cystine.

A possible explanation of the continued weight loss in the face of a positive nitrogen balance seen in dogs 3 and 5 may be the shift in liquid balance with a decrease in available body

[3] Personal communication from Dr. J. B. Allison.

fluid as the hypoproteinemia regressed. The dogs were hypoproteinemic throughout the study, however, and this condition was not clearly corrected by the low level of nitrogen given. It appears that the level at which a protein material will support nitrogen balance is not always a safe criterion as to the appropriate dietary level of the material for long-term maintenance, especially if equilibrium is approached from the negative side. Weight balance should also be considered as well as the general health of the animal.

The complete failure of unfortified casein hydrolysates to support nitrogen balance at levels up to 160 mg N per kilo per day is apparent from the work with dogs 7, 8, and 12. The inadequacy of casein hydrolysate with added cystine at about 1.6% is indicated from the records of dogs 10 and 11. Analysis for the essential amino acids in casein and fibrin hydrolysates (Frost, Heinsen and Olsen, '46) indicated the cystine contents to be about 0.6 and 2.2% and the methionine contents to be about 2.5 and 1.8%, respectively, on a dry basis.[4] Thus, in those instances in which cystine was added, the casein hydrolysate was as rich in cystine and richer in methionine than the fibrin hydrolysate.

The superiority of partial acid hydrolysates of fibrin over those of casein has been further demonstrated in rat growth studies in this laboratory.[5] At 18% of the diet, dried partial acid hydrolysate of fibrin gave growth equal to that obtained with 18% casein, whereas casein hydrolysate produced only negligible growth. The addition of cystine or methionine to casein or dried casein hydrolysate produced a marked growth response in rats whereas addition of these amino acids to fibrin hydrolysate gave only minor stimulation of the growth rate. Risser ('46) previously demonstrated the marked deficiency effect of sulfur amino acids in casein fed orally to dogs. It was also clear from his studies that fibrin contained an adequate complement of cystine and methionine.

[4] The methionine, cystine, and tryptophane determinations were made by Mr. E. O. Krueger.

[5] The rat growth experiments were conducted by F. Peirce Dann.

Maintenance of nitrogen balance in intravenous studies with dogs (Risser, Schenck and Frost, '46a, '46b) with casein hydrolysate was accomplished only with a high cysteine supplement and the periods of injection were relatively short as compared with present studies. Kade, Houston, Krauel and Sahyun ('46) maintained dogs in positive nitrogen balance on 214–230 mg N per kilo per day in the form of a complete hydrolysate of casein fortified with 1% dl-tryptophane given intravenously for 1-week periods. They state that this level is near minimal; however, no supplement of sulfur amino acids was made in their studies.

The levels of sulfur amino acids are nearly equalized between the two protein hydrolysates in the present studies, but in view of the earlier results, the casein hydrolysate still appears deficient in sulfur amino acids. The possibility appears, therefore, that the cystine and/or methionine of casein and partial hydrolysates of casein is not completely utilized. This possibility is being studied further.

A basis for difference in utilization of the partial acid hydrolysates of casein and fibrin also resides in the wide difference in tryptophane content, i.e., about 0.55 and 1.1% for casein and fibrin, respectively. Risser, Schenck and Frost ('46b) indicated, however, in short term studies that the relatively low level of tryptophane in the casein hydrolysate is probably not critical. In view of the many variables involved in this type of study exact deductions as to critical levels of the essential amino acids are difficult to make and will require extensive study under a variety of conditions. There are, of course, other bases for difference in utilization between fibrin and casein hydrolysates given intravenously. Chief among these is the factor of peptide utilization about which very little is known.

SUMMARY

Two dogs were maintained for 15 weeks on fibrin hydrolysate (acid hydrolysate) and fibrin hydrolysate plus dextrose intravenously at levels ranging from 85–120 mg

nitrogen per kilo per day. Nitrogen balance became positive when the level was raised to 110 mg nitrogen per kilo, although weight loss continued. The nitrogen balance index for fibrin hydrolysate given intravenously was calculated to be 0.83–0.86.

Partial acid hydrolysates of casein, alone or with an isocaloric addition of dextrose, failed to maintain nitrogen balance when given intravenously in four dogs at levels up to 160 mg nitrogen per kilo per day. Addition of 1.6% cystine on a dry basis failed to improve the casein hydrolysate markedly under the conditions of the experiments.

ACKNOWLEDGMENT

Acknowledgment is gratefully made to Robert T. Olsen and Oscar Walther for technical administration of the hydrolysates and to Mr. H. C. Spruth and Mr. E. B. Vliet for departmental cooperation in making the work possible.

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VITAMIN B COMPLEX STUDIES WITH DIETS DIFFERING IN THE CARBOHYDRATE COMPONENT [1]

HELEN R. SKEGGS AND LEMUEL D. WRIGHT

Nutritional Laboratory, Department of Pharmacology, Medical Research Division, Sharp and Dohme, Glenolden, Pennsylvania

ONE FIGURE

(Received for publication June 6, 1946)

Highly purified diets containing sucrose or dextrin, supplemented with all of the recognized B vitamins except folic acid and biotin, support excellent growth of rats. Such diets are inadequate, however, when poorly absorbed sulfonamides are included and a deficiency syndrome develops that may be overcome by the administration of solubilized liver or folic acid and biotin (Daft et al., '42; Nielsen and Elvehjem, '42; Welch and Wright, '43; Schweigert et al., '45). It is probable that in the absence of poorly absorbed sulfonamides the requirement of the rat for folic acid and biotin is satisfied by intestinal synthesis. The studies of previous investigators, recently reviewed by Najjar and Barrett ('45), have indicated that bacterial synthesis of the B vitamins in the intestine may be materially influenced by the composition of the diet. Changes in the carbohydrate component of the diet, for example, have been shown to alter both the intestinal synthesis of certain B vitamins and the dietary requirement thereof. The experiments reported in this paper were undertaken in an effort to obtain additional information on the role of the carbohydrate component and the effect of succinylsulfathiazole (SST) in vitamin B synthesis and utilization by the rat.

[1] Presented before the American Institute of Nutrition, March, 1946. (Federation Proceedings, vol. 5, p. 240, 1946).

EXPERIMENTAL

Weanling male albino rats weighing about 40 gm were used. There were four rats in each group that received purified diets containing sucrose, cerelose, dextrin, corn starch, or lactose. Six rats in each group received the same carbohydrate diets containing 2% SST. The diets employed were composed of carbohydrate, 618 gm; casein, 180 gm; salt mixture (Hubbell, Mendel and Wakeman, '37), 40 gm; cellu flour, 40 gm; choline chloride, 1 gm; "Primex," 100 gm; corn oil, 20 gm; A-D-E concentrate (Wright et al., '45), 1 gm; "Menadione," 10 mg; thiamine hydrochloride, 8 mg; riboflavin, 16 mg; nicotinic acid, 40 mg; pyridoxin hydrochloride, 8 mg; calcium pantothenate, 44 mg; p-aminobenzoic acid, 40 mg; and inositol, 216 mg. When succinylsulfathiazole was included in the diet it replaced 2% of the weight of the carbohydrate employed. Records were kept of the appearance and weight changes of all the animals, and, at intervals, of the food consumption. Determinations of the folic acid, biotin, pantothenic acid, nicotinic acid, and riboflavin content of the feces were made at weekly intervals. The pooled samples from all of the rats in each group, collected over a 24-hour period, were divided into two portions. One portion was digested with 2% of its weight of takadiastase in 1% acetate buffer at pH 4.0 under benzene for 24 hours (Cheldelin et al., '42), prior to assay for folic acid (Landy and Dicken, '42), pantothenic acid (Skeggs and Wright, '44), nicotinic acid (Snell and Wright, '41) and riboflavin (Snell and Strong, '39). The other portion was autoclaved in 10 ml of 6 N H_2SO_4 for 1 hour at 120°C. prior to microbiological assay for biotin (Wright and Skeggs, '44). The bacterial flora of the feces were followed at weekly intervals by the dilution-plate count method of Strawinski et al. ('46). White blood cell and differential counts were made at intervals throughout the feeding period on blood obtained by clipping the tail, and at the conclusion of the experiment, when the rats were sacrificed, on blood obtained following decapitation. The livers were removed from all of the animals immediately following decapitation and were divided into two

parts. One part was digested with 2% of its weight of takadiastase in 20 ml of water under benzene prior to microbiological assay for folic acid, pantothenic acid, nicotinic acid and riboflavin. The other part was autoclaved for 1 hour at 120°C. in 10 ml 6 N H_2SO_4 prior to assay for biotin.

RESULTS

In the absence of SST all of the diets except that containing lactose promoted good to excellent growth and appearance of the rats (fig. 1). The greatest weight gains occurred in rats receiving corn starch, with the least gains in the rats ingesting sucrose. The animals fed lactose developed diarrhea, lost weight, and failed to survive. No attempt has been made to summarize the limited growth data obtained on lactose diets.

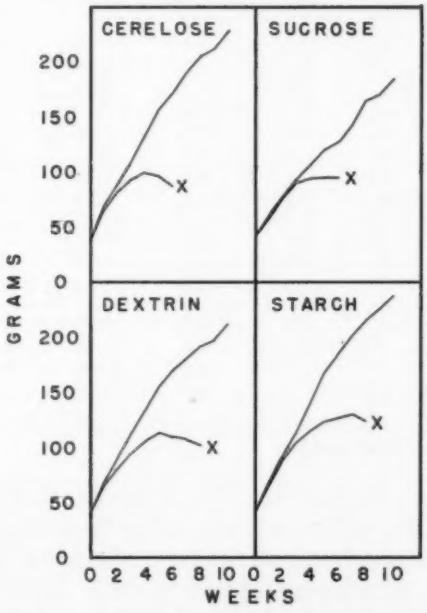

Fig. 1 Growth curves of the rats receiving the various carbohydrate diets. In each instance the upper and lower curves represent, respectively, the results obtained with the basal diet and the corresponding SST-containing diet.

The inclusion of SST in diets containing sucrose, cerelose, dextrin, or corn starch resulted in poor growth and early death. Irrespective of the carbohydrate fed the rats receiving SST developed alopecia, porphyrin-stained whiskers, sore eyes and rough coats.

The food intake studies (table 1) indicated that the poor growth resulting when SST was included in the diet was due

TABLE 1

Summary of food intake data.

CARBOHYDRATE	WEEKS ON TEST	NUMBER OF SAMPLES	FOOD INTAKE			
			No succinylsulfathiazole		2% succinylsulfathiazole	
			Average intake	Range	Average intake	Range
			gm	*gm*	*gm*	*gm*
Sucrose	4	12	9.4	7–11	6.3	3–10
	5	12	9.8	6–13	5.1	1– 8
	7	16	10.7	6–15	5.9	0–11
Dextrin	4	12	10.7	7–14	7.9	6– 9
	5	12	11.7	10–15	6.6	4–10
	7	16	11.6	9–15	5.1	2– 8
	8	12	12.3	10–15	4.8	1–14
Cerelose	4	12	12.2	10–15	7.3	2–11
	5	12	13.4	8–16	7.3	4–12
	7	16	12.9	6–16	6.6[1]	0–12
Corn starch	4	12	12.2	9–14	9.6	7–11
	5	12	13.1	10–16	8.8	6–12
	7	16	13.2	11–17	7.9	5–11
	8	12	13.7	11–17	9.3	1–14

[1] Average of 10 determinations.

in part to loss of appetite. The sulfonamide-fed rats generally ate less, and as the deficiency signs became more apparent, certain animals refused all the food offered to them.

The fecal elimination of the various factors, when considered as the amount of each factor excreted per rat in 24 hours, varied with the diet (table 2). On this basis the dextrin-fed rats eliminated appreciably more of all of the factors

TABLE 2 [1]

Fecal elimination of B vitamins.

DIET	NO. OF DET'NS.	FOLIC ACID		BIOTIN		PANTOTHENIC ACID		NICOTINIC ACID		RIBOFLAVIN	
		μg/rat/day	μg/gm feces	μg/rat/day	μg/gm feces	μg/rat/day	μg/gm feces	μg/rat/day	μg/gm feces	μg/rat/day	μg/gm feces
Sucrose	9	4.7 (1.5–6.3)	3.9 (2.5–4.6)	0.39 (0.22–0.48)	0.34 (0.22–0.44)	26 (13–31)	23 (18–29)	31 (16–38)	29 (18–43)	18 (9–27)	17 (12–24)
Sucrose + SST	8	0.90 (0.38–1.4)	1.2 (0.6–1.7)	0.32 (0.19–0.43)	0.42 (0.24–0.56)	17 (13–22)	22 (19–29)	29 (19–38)	35 (33–44)	11 (7–15)	14 (10–21)
Dextrin	9	6.7 (4.9–10.0)	4.7 (3.2–6.5)	1.6 (0.95–2.1)	1.2 (0.78–1.24)	71 (54–106)	50 (26–63)	108 (96–124)	78 (55–91)	23 (15–28)	17 (15–22)
Dextrin + SST	9	1.6 (0.86–2.3)	1.3 (1.0–1.6)	1.2 (0.51–1.5)	1.0 (0.30–1.26)	40 (24–63)	32 (26–37)	93 (50–128)	75 (62–95)	28 (13–43)	23 (17–33)
Cerelose	9	4.1 (2.0–6.7)	3.3 (2.2–4.1)	0.62 (0.46–0.85)	0.50 (0.35–0.62)	27 (10–32)	23 (14–34)	27 (21–35)	22 (17–30)	12 (7–21)	10 (7–13)
Cerelose + SST	8	1.5 (0.85–2.3)	1.5 (1.3–2.2)	0.37 (0.24–0.47)	0.40 (0.27–0.51)	20 (11–30)	21 (16–24)	29 (15–47)	30 (24–39)	10 (5–18)	10 (8–13)
Corn starch	9	3.9 (3.1–6.9)	3.2 (2.3–4.0)	0.75 (0.47–1.0)	0.64 (0.52–0.78)	30 (22–38)	25 (19–32)	35 (22–51)	29 (21–31)	11 (6–17)	10 (6–12)
Corn starch + SST	9	1.4 (0.70–2.7)	1.1 (0.7–1.6)	0.44 (0.28–0.61)	0.39 (0.28–0.47)	23 (19–34)	21 (17–26)	32 (27–38)	30 (24–36)	11 (7–14)	9 (7–15)

[1] The figures in parentheses in this and subsequent tables indicate the range in values obtained.

studied than did the animals on the other carbohydrate diets. The elimination of folic acid was markedly lower and that of biotin and pantothenic acid slightly lower when SST was included in the diets. Nicotinic acid and riboflavin elimination was not affected by the drug. However, the food intake studies have shown that less food was consumed by the rats ingesting SST. Since the amount of feces excreted is somewhat dependent on the amount of food consumed, it may be that the growth factors eliminated per gram of fecal material constitute a more accurate basis for a comparison of bacterial synthesis promoted by the various diets. On this basis the synthesis of folic acid per gram of feces was definitely less when SST was included in all of the carbohydrate diets. The decrease in the elimination of biotin and pantothenic acid in most cases, probably was caused, not by decreased synthesis or increased destruction of these factors, but by a diminution in the intestinal bulk, since the incorporation of SST in the diet was accompanied by decreased food intakes. The increased production of the various factors by the dextrin-fed rats would appear to be caused by actual stimulation of intestinal synthesis rather than merely by an increase in the amount of material passing through the intestine.

The variations found in the fecal elimination of the various factors cannot be explained by obvious changes in the intestinal flora. The inclusion of SST, irrespective of the carbohydrate in the diet, resulted in a decrease in the number of coliforms present, but neither the drug nor the carbohydrate exerted any other significant effect on the type or number of organisms found in the feces. That the actual number of organisms present, however, is no criterion of the capacity for synthesis of the B vitamins has been shown by the in vitro studies of Miller ('44) who found that, while cultures of Escherichia coli may attain equal turbidity (i.e., equal numbers of organisms), those organisms grown in the presence of sulfanilamide produce less folic acid than those grown in a similar medium containing no drug.

TABLE 8

Summary of white blood cell and differential leucocyte counts (cells per mm³)

TABLE 3

Summary of white blood cell and differential leucocyte counts (cells per mm³).

CARBOHYDRATE	DAYS ON TEST	NO SUCCINYLSULFATHIAZOLE			2% SUCCINYLSULFATHIAZOLE		
		No. of det'ns.	Total white count	Total neutrophils	No. of det'ns	Total white count	Total neutrophils
Sucrose	42	4	20,600 (10,300–29,000)	2,350 (1,100–4,000)	6	8,600 (4,400–12,500)	706 (152–2,080)
	56	4	18,100 (12,200–25,100)	3,860 (2,190–4,750)	4	4,100 (3,000–5,200)	245 (162–354)
	63	4	8,400 (6,200–13,600)	1,150 (830–1,860)			
Dextrin	42	4	18,700 (10,800–24,000)	2,230 (1,560–3,120)	6	9,800 (4,500–15,800)	630 (316–1,930)
	63	4	18,300 (14,300–25,900)	2,940 (2,400–3,220)	4	3,300 (1,100–5,100)	172 (32–278)
	70	4	8,500 (4,300–12,800)	1,060 (610–1,830)			
Cerelose	42	4	17,800 (14,200–20,200)	1,970 (1,200–2,600)	6	8,000 (3,400–13,700)	780 (85–1,890)
	56	4	20,100 (17,600–23,300)	2,200 (1,820–2,720)	2	3,300 (3,200–3,300)	97 (13–180)
	70	4	6,800 (5,000–11,500)	790 (450–1,260)			
Corn starch	42	4	16,700 (12,100–21,600)	1,740 (850–2,780)	6	9,000 (4,200–11,900)	671 (85–980)
	63	4	21,500 (20,300–24,200)	2,970 (1,560–4,500)	5	4,700 (2,100–6,800)	356 (195–945)
	70	4	8,100 (6,300–8,800)	940 (720–1,140)			

TABLE 4

Hepatic storage of B vitamins.

DIET	DAYS ON TEST	NO OF DET'NS.	FOLIC ACID	BIOTIN	PANTOTHENIC ACID	NICOTINIC ACID	RIBOFLAVIN
			μg/gm	μg/gm	μg/gm	μg/gm	μg/gm
Sucrose	70	4	0.42 (0.39–0.50)	0.42 (0.31–0.52)	87 (82–96)	178 (161–192)	30 (27–31)
Sucrose + SST	56	4	0.25 (0.18–0.34)	0.28 (0.19–0.40)	55 (42–75)	182 (135–217)	31 (26–39)
Dextrin	70	4	0.55 (0.44–0.81)	0.99 (0.65–1.3)	211 (161–234)	166 (154–175)	33 (29–35)
Dextrin + SST	63	4	0.49 (0.15–0.66)	0.64 (0.55–0.79)	76 (51–103)	160 (120–187)	31 (26–35)
Cerelose	70	4	0.61 (0.38–0.98)	0.72 (0.55–0.81)	115 (98–135)	170 (155–190)	32 (29–38)
Cerelose + SST	56	2	0.26 (0.13–0.39)	0.26 (0.24–0.28)	63 (62–65)	146 (129–163)	26 (25–27)
Corn starch	70	4	0.38 (0.35–0.41)	0.63 (0.46–0.83)	136 (102–159)	179 (158–200)	30 (24–33)
Corn starch + SST	63	4	0.22 (0.17–0.28)	0.30[1] (0.17–0.39)	59[1] (46–66)	163[1] (146–176)	28 (26–32)

[1] Average of 5 determinations.

A summary of the white blood cell data is given in table 3. The leucopenia and granulocytopenia characteristic of sulfonamide-induced folic acid deficiency were found in all of the rats fed SST, irrespective of the type of carbohydrate in the diet. The control rats on each carbohydrate diet had a normal leucocyte picture when the SST-fed rats were sacrificed. The terminal counts on the control groups, however, generally were lower with respect to both total and neutrophil counts than was anticipated. It was felt that the low terminal counts might be indicative of an incipient folic acid deficiency. Kornberg and his associates ('45) have reported that a small percentage of rats ingesting purified diets over a long period of time do develop leucopenia and granulocytopenia that may be alleviated by the administration of folic acid.

It was reported previously (Wright et al., '45) that a level of approximately 0.5 γ or less of folic acid per gram of liver is associated with evidences of a folic acid deficiency in rats. From table 4 it may be seen that in general the rats receiving SST had an hepatic storage characteristic of a folic acid deficiency. The hepatic storage of folic acid in rats ingesting the various carbohydrate diets without drug was approaching the level previously described as critical, confirming the tendency toward a folic acid deficiency suggested by the low leucocyte counts. The liver storage of biotin and pantothenic acid was depressed by the inclusion of SST in all the carbohydrate diets. Nicotinic acid and riboflavin were found in the liver in normal amounts regardless of the carbohydrate or drug in the diet. The storage of pantothenic acid in the livers of rats fed the control dextrin diet was remarkably high. Because of the large amounts of folic acid, biotin, nicotinic acid and pantothenic acid eliminated in the feces of the dextrin-fed rats, an increased liver storage of all of these factors might have been expected. Actually pantothenic acid was the only factor to be stored in unusually large amounts.

DISCUSSION

These studies have demonstrated that the type of dietary carbohydrate used in highly purified diets is of little significance in the production by SST of a combined folic acid, biotin and, frequently, pantothenic acid deficiency. The study made of food consumption and fecal elimination of the various factors has clarified, to a large extent, the role of SST in relation to the intestinal synthesis of essential factors. The in vitro studies of Miller ('44) have shown that the presence of subbacteriostatic amounts of sulfanilamide in cultures of Escherichia coli depresses the ability of the organism to synthesize folic acid. This principle apparently applies in vivo. The administration of SST to the rat does not alter appreciably the intestinal flora, except for a reduction in the number of coliforms, but does act directly to decrease the synthesis of folic acid. Animals subsisting on purified diets that do not contain poorly absorbed sulfonamides have at best a marginal hepatic storage of folic acid and biotin. Since the only source of these factors in such diets is that produced by intestinal synthesis, the action of SST is partially to block off the source of supply and the rats develop a folic acid deficiency. As the animals become deficient, food intakes tend to diminish. The data have shown that the amount of biotin produced per gram of feces generally is not altered by the administration of the drug. Through decreased food intake, however, the intestinal bulk is diminished and consequently less biotin becomes available to the animal for growth. Depletion of the small amount of biotin stored in the tissues ensues. At present, the frequent instances of pantothenic acid deficiency are more difficult to explain. Although decreased food consumption as a result of the combined folic acid and biotin deficiencies is undoubtedly a factor, other lines of evidence indicate that this is not a complete explanation.

SUMMARY

1. A study has been made of the growth, food consumption, intestinal flora, leucocyte picture, fecal elimination and

hepatic storage of folic acid, biotin, pantothenic acid, nicotinic acid and riboflavin of rats receiving highly purified diets containing glucose, sucrose, lactose, dextrin or corn starch as the carbohydrate component, with and without the addition of succinylsulfathiazole (SST).

2. The inclusion of SST in highly purified diets irrespective of the carbohydrate component is effective in producing a combined folic acid and biotin deficiency.

3. The intestinal synthesis of folic acid, and consequently the amount available to the animals, is depressed directly by the administration of SST.

4. The feeding of purified diets containing no drug promotes the synthesis of sub-optimal amounts of folic acid since prolonged feeding of such diets may result in hepatic stores of folic acid indicative of an incipient deficiency.

5. In the absence of SST, the carbohydrate component of the diet, as in the case of dextrin, may influence the synthesis of certain B vitamins in the intestine of the rat, and indirectly the tissue storage of pantothenic acid.

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FURTHER EXPERIMENTS ON THE RELATION OF FAT TO ECONOMY OF FOOD UTILIZATION

I. BY THE GROWING ALBINO RAT [1]

E. B. FORBES, R. W. SWIFT, W. H. JAMES, J. W. BRATZLER AND ALEX BLACK

Institute of Animal Nutrition, The Pennsylvania State College, State College

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In a recent number of this journal Forbes, Swift, Elliott and James ('46) published results of experiments showing that with growing rats on diets containing 2, 5, 10 and 30% of fat, respectively, and so compounded and fed as to supply identical quantities of energy and of protein, the gains in live weight, the digestibility of nitrogen, and the retention of nitrogen and energy were in the order of the increasing fat contents of the diets; and that with mature rats on the same diets as above specified the energy expense of utilization (dynamic effects) of these diets diminished in the order of the increasing fat contents of the diets.

In the conduct of these experiments it was the intent of the authors to compound the diets in such manner as to supply all nutrients required for the most efficient utilization of food energy; but it is conceivable that the vitamin requirements were not completely satisfied.

It was decided, therefore, to repeat these experiments with three of the diets, those containing 2, 10 and 30% of fat, with large increases in ten of the vitamins.

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EXPERIMENTAL

In the repetition of these experiments the diets with both the growing and the mature rats were composed as is shown in table 1, the results with growing rats being given in the present paper and those with mature rats in the paper which follows. Though the present paper presents results of the experiments of 1945–46 alone, the amounts of the vitamins supplied as supplements to the diets of this year, and of the preceding year as well, are given for comparison in table 2.

In both years' experiments it was assumed that the requirements of the rats for essential fatty acids were satisfied by the corn oil, and that the requirements for vitamin D, biotin and folic acid were satisfied by the yeast component of the diets.

The quantitative basis for increasing the intake of vitamins in the experiments of 1945–46 as compared with those of 1944–45 was largely empirical. The increase in the amount of choline was suggested by C. A. Elvehjem (letter of September 22, 1945); and H. E. Longenecker suggested (letter of September 27, 1945) that the amounts of pyridoxine and of pantothenic acid provided in the experiments of 1944–45 might have been at borderline levels. The amounts by which the allowances of the other vitamins were increased, however, were determined in a purely arbitrary manner.

The method of computation of the makeup of the diets as required to supply fat at three levels, with protein constant, and with carbohydrate varying reversely as the fat, in order to provide a constant energy intake, will be given.

Referring to table 1, it will be noted that with the salt mixture and the corn oil as constants, the three levels of fat intake were provided by the introduction of lard into diets no. 2 and 3. The protein and the carbohydrate mixtures were arbitrarily devised; and the amounts supplied of each, in order to provide protein at a constant level, and carbohydrate in the quantities required to maintain a constant energy intake in the three diets, were computed by an algebraic process which will be explained. The nitrogen content of the protein

mixture was 11.05%; and the energy values of the dietary components, as determined by the bomb calorimeter, were as follows: protein mixture 4825 cal., carbohydrate mixture 3697 cal., corn oil 9382 cal., and lard 9431 cal., per gm. These values are the average of duplicate or triplicate determinations which differed among themselves by less than 1%.

Diet no. 1 was arbitrarily made up to contain 4% salt mixture, 2% corn oil, and 22% protein. Using this combination,

TABLE 1

General composition of diets.

COMPONENTS	DIET NO. 1	DIET NO. 2	DIET NO. 3
	%	%	%
Salt mixture [1]	4.00	4.00	4.00
Corn oil [2]	2.00	2.00	2.00
Protein mixture [3]	31.86	35.84	45.82
Carbohydrate mixture [4]	62.14	50.16	20.18
Lard [5]	0.00	8.00	28.00
Cal. per gm	4.02	4.526	5.785
Isocaloric factors	1.0000	0.8887	0.6953

[1] U.S.P. XII no. 2.

[2] The energy of the corn oil was 9.382 Cal. per gm.

[3] Casein 70%, skim milk powder 15%, irradiated yeast 10%, and brewer's yeast 5%. Mixture contained 11.05% N, and 4.825 Cal. per gm.

[4] Corn starch 34%, sucrose 33%, dextrin 12%, and dextrose (cerelose) 21%. Mixture contained 3.697 Cal. per gm.

[5] The energy of the lard was 9.431 Cal. per gm.

TABLE 2

Vitamins added per kg of diet 1 and to isocaloric quantities of other diets in experiments of 1944–45 and 1945–46.

	1944–1945	1945–1946		1944–1945	1945–1946
	mg	*mg*		*mg*	*mg*
Carotene	6.0	40	Choline chloride	400	2000
Thiamine hydrochloride	5.0	20	Alpha-tocopherol	100	200
Riboflavin	5.0	20	Inositol	2000	2000
Pyridoxine hydrochloride	6.25	20	Para-aminobenzoic acid	95	200
Niacin	6.25	20	2-methyl-1, 4 naphtho-quinone	2.5	6
Calcium pantothenate	50	100			

the theoretical amount of protein mixture required per 100 gm of the diet was found to be 31.8552 gm, leaving 62.1448 gm to be provided by the carbohydrate mixture, the energy value of the whole being 4022 cal. per gm, and the ratio of the energy value of the protein mixture to the energy value of the complete diet being as 0.3821:1. It then remained to compute the other diets in such manner as to contain 10% and 30% of fat, respectively, and to provide protein and energy in the same quantities as supplied by diet no. 1. To compute the 10%-fat diet, the composition of diet no. 1 was modified by adding 8% of lard to the 2% of corn oil and 4% of salt mixture, leaving 86% of the diet to be provided by the protein and carbohydrate mixtures.

If x represented the percentage of protein mixture per 100 gm of diet no. 2, and 86–x represented the percentage of carbohydrate mixture, then

$$\frac{4825\,x}{4825\,x + (86-x)\,3697 + 2\,(9382) + 8\,(9431)} = 0.3821$$

This gave a percentage of 35.84 for x, and left a percentage of 50.16 for the carbohydrate mixture. These factors were set up on the calculating machine. When the rations were made up (3 times during the experiment), the amounts of all five components listed in table 1 were weighed to an accuracy of 1 gm.

The energy value of this diet was 4526 cal. per gm, and its protein content was 24.76%. The ratio of the energy of equal quantities of diet no. 1 to diet no. 2, as well as the ratio of their protein contents was as 1 to 0.889. Therefore, for every gm of diet no. 1 there was fed 0.889 gm of diet no. 2, which supplied equal amounts of protein and energy — the only difference being in the sources of the nonprotein energy. The makeup of the 30%-fat diet was computed by the same procedure; and the results of these computations are given in table 3. Daily feeds were weighed on a chainomatic balance sensitive to 0.1 mg.

Since the diets of different fat contents also differed in their carbohydrate contents it is necessary to stress the fact that it would be as logical to relate the differences in their nutritive value to the differences in carbohydrate as to the differences in fat. However, the results of this experiment are discussed in relation to the fat contents of the diets for the sake of convenience, and because of interest in the physiological effects and dietetic relationships of fat.

As in the earlier experiment with growing rats, the subjects in the present experiment were allowed normal freedom of movement. The method of experimentation provided for the computation of a single measurement of the heat production for a 70-day period as the gross energy of the feed minus

TABLE 3

Constituents of diets of different fat and different carbohydrate contents which supply the same quantities of protein and energy.

DIET NO.	APPROXIMATELY ISOCALORIC QUANTITIES OF DIETS	PROTEIN		FAT		CARBOHYDRATE		ENERGY	
	gm	*%*	*gm*	*%*	*gm*	*%*	*gm*	*cal./gm*	*cal.*
1	10.00	22.00	2.20	2.00	.20	65.26	6.53	4022	40.22
2	8.89	24.76	2.20	10.00	.89	54.91	4.88	4526	40.24
3	6.95	31.64	2.20	30.00	2.09	28.68	1.99	5785	40.21

the energy of the excreta and of the body gain. The experiments with mature rats as subjects, with voluntary activity excluded by close confinement in respiration chambers, were conducted by the open-circuit Haldane procedure, the heat increment or dynamic effect of the diets being determined as the difference in the quantities of heat produced from a basal quantity of food sufficient for maintenance and a larger quantity of the same diet.

The experiment with growing rats involved thirty-six male animals, three rats being taken from each of twelve litters. In each group of three litter mates, each rat received one of the three diets. The animals were 24 to 27 days old at the

beginning of the experimental feeding and 70 days older when they were killed for analysis at the end of the experiment. The food eaten during the 70 days was 773 gm of diet no. 1, and isocaloric quantities of diets no. 2 and 3.

During this test the daily urines were made up to 250 ml, from which a 25 ml aliquot was put into a 2.5 liter bottle in which the urine was preserved with sulphuric acid, and the feces, as collected daily, were dried in the air at room temperature. The energy of the urine was taken at 8.6 $\pm$ 0.05 Cal. per gm N. This factor was derived from comparable experiments of the previous year in which similar diets were fed,

TABLE 4

Average liveweights of rats during 10 weeks on isocaloric quantities of diets containing 2, 10 and 30%, respectively, of fat.

DIET NO.	FAT CONTENTS OF DIET	INITIAL BODY WEIGHT	WEEK NUMBER									
			1	2	3	4	5	6	7	8	9	10
	%	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm	gm
1	2	42	59	88	118	145	173	199	224	245	262	274
2	10	42	59	87	117	144	174	200	227	249	266	278
3	30	42	58	87	116	142	171	200	227	249	268	280

and the energy of the urine was determined by the bomb calorimeter.

The average weekly liveweights recorded in table 4 indicate a conceivable advantage in favor of the 2%-fat diet as compared with the 30%-fat diet of 1 gm in the first week, increasing to 3 gm in the fourth, and to 2 gm in the fifth week. Between the fifth and sixth weeks, however, this hypothetical advantage in favor of the low-fat diet disappeared, the weights for the sixth to the tenth weeks indicating an advantage in favor of the 30%-fat diet as compared with the 2%-fat diet of 1, 3, 4, 6 and 6 gm, respectively.

The computed odds that the average liveweight of the rats on the 30%-fat diet were significantly higher than the weight of the rats on the 2%-fat diet were only 11 to 1. The observed differences in average liveweight of the rats which

received the different diets, therefore, were not statistically significant.

It is shown in table 5 that the body gains of nitrogen of the rats diminished, and the gains of fat increased, in the order of the increasing fat contents of the diets, the odds of significance between the 2%- and the 30%-fat diets being 15:1 for the nitrogen retention and 151:1 for the fat gains.

On the other hand, in the earlier experiment there were significant increases in nitrogen retention in the order of the increases in the fat contents of the diets.

TABLE 5

Average amounts of food eaten during 70 days; and initial and final nitrogen and fat contents of rat bodies.

FAT CONTENTS OF DIETS	FOOD EATEN	INTAKE OF NITROGEN	BODY NITROGEN		BODY FAT	
			Initial	Final	Initial	Final
%	gm	gm	gm	gm	gm	gm
2	780.0	27.46	1.02	8.97	3.23	29.76
10	693.2	27.46	1.02	8.83	3.25	34.36
30	542.3	27.46	1.02	8.67	3.24	39.66

It is unnecessary to attempt a fundamental interpretation of this difference in evidence relating to nitrogen utilization, first because it was not technically positive, and second because the relative proportions of protein, carbohydrate and fat catabolized may vary considerably, as influenced by superficial environmental conditions, without such variation being of fundamental significance.

It is shown in table 6 that the urine nitrogen increased, and the fecal nitrogen, as well as the nitrogen retained, decreased, without exception, in the order of the increase in the fat contents of the diets, but the significance of these observations is not to be stressed — for the reasons given above.

It is noteworthy that the nitrogen recovery during the feeding experiment, as indicated by the quantity of nitrogen in the excreta, plus the quantity of nitrogen retained (as deter-

mined by body analysis), divided by the quantity of the nitrogen intake, was 99% or more in each case.

In table 7 is given the partition of the average daily intake of food energy per rat during 70 days.

The decrease in carbohydrate intake compensating for the increasing fat content of the diets was without positive effect

TABLE 6

Partition of average nitrogen intake per rat during 70 days.

FAT CONTENT OF DIETS	NITROGEN				
	Intake	Output in		Retained [1]	Retained [2]
		Urine	Feces		
%	*gm*	*gm*	*gm*	*gm*	*gm*
2	27.46	17.36	1.89	8.21	7.95
10	27.46	17.61	1.76	8.09	7.81
30	27.46	18.03	1.53	7.90	7.65

[1] Nitrogen in the food minus nitrogen in the excreta.

[2] Nitrogen in the bodies of the rats which were fed for 70 days minus the nitrogen in the bodies of the control group.

TABLE 7

Partition of average daily intake of food energy per rat during 70 days.

FAT CONTENT OF DIETS	GROSS ENERGY INTAKE	ENERGY INTAKE				ENERGY OUTPUT			ENERGY RETAINED
		Protein	Carbohydrate	Fat	Metabolizable	In feces	In urine	As heat	
%	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
2	3137	978	2011	148	2872	116	149	2333	539
10	3137	978	1501	658	2844	142	151	2266	578
30	3137	978	614	1545	2815	167	155	2195	620

on the metabolizable energy of the diet. The three factors of energy outgo, however, present a highly significant picture of the effect of food fat on the efficiency of utilization of food energy. Thus, in spite of marked increase in the energy of the feces, and the nonsignificant increase in the energy of the urine, there was a definite decrease in the heat production

(odds 1999:1), and an increase in energy retention (odds 98:1), in the order of the increasing fat contents of the diets.

In the light of present knowledge and evidence, therefore, fat confers efficiency of utilization of food energy for the growing albino rat.

The evidence to this effect was more positive in the present experiment, with large increases in the intake of ten of the vitamins, than in the earlier work from this laboratory, to which the authors have referred, in which a lower level of vitamin intake prevailed.

SUMMARY

A 70-day metabolism and body analysis experiment was conducted to determine the effects of differences in the fat content of diets, with large increases in the intake of ten of the vitamins, as compared with earlier experiments, on the utilization of food energy and protein by growing albino rats.

The subjects were three groups of twelve weanling males, each group containing one rat from each of the same twelve liters.

A comparison was made between three diets containing 2, 10 and 30% of fat, respectively, these diets being so compounded and fed as to supply to each rat of a litter-three, and therefore to each group of 12, the same quantities of energy, protein, and vitamins.

Determinations were made of gains in live weight, nitrogen, fat and energy, and of the heat production for 70 days as the energy of the food minus the energy of the excreta and of the body gain.

The statistically significant results were body gains of fat and energy, and decrease in the heat production, in the order of the increasing fat contents of the diets.

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FURTHER EXPERIMENTS ON THE RELATION OF FAT TO ECONOMY OF FOOD UTILIZATION

II. BY THE MATURE ALBINO RAT [1]

E. B. FORBES, R. W. SWIFT, E. J. THACKER, VIVIAN FRANCES SMITH AND C. E. FRENCH

Institute of Animal Nutrition, The Pennsylvania State College, State College

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This experiment was a repetition of the greater part of an earlier study by Forbes, Swift, Elliott and James ('46), with greatly increased intake of ten of the vitamins, for the purpose of learning whether the results observed in the former experiment, also with mature rats, were determined to any extent by an intake of less than optimum amounts of these nutrients. The specific object was to study the energy and nitrogen metabolism of isocaloric quantities of diets containing 2, 10 and 30% of fat, respectively, each supplying the same quantity of protein, and each fed at two levels of intake.

For convenience, the two planes of nutrition providing for the relation of the difference in food to the difference in heat production were designated "maintenance" and "supermaintenance," the energy of the higher plane of intake being 45.45% greater than that of the lower plane.

As a matter of fact, however, the exact maintenance requirements of the rats were not determined in the respiration experiments since the metabolizable energy was determined while the animals were free to move about, and the heat production was determined with voluntary activity excluded.

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The general experimental routine was exactly the same as in the earlier study referred to.

The subjects were three groups of twelve essentially mature male albino rats about 6 months old. These three groups of rats were born at intervals of time which made all three groups equal in age when used in the respiration experiments.

The diets were composed as shown in table 1 of the foregoing paper on growing rats (page 399). As shown in table 1 of the present paper, the rats on the 2%-fat diet received 11 and 16 gm each of food at the two planes of nutrition,

TABLE 1

Percentage contents of diets and daily intake of protein, carbohydrate, fat and energy.

DIET NO.	PLANE OF NUTRITION	TOTAL FOOD INTAKE	PROTEIN		CARBO-HYDRATE		FAT		ENERGY
		gm	%	*gm*	%	*gm*	%	*gm*	*Cal.*
1	Maintenance	11.00	22.00	2.42	65.26	7.18	2.00	0.22	44.24
2	Maintenance	9.78	24.75	2.42	54.92	5.37	10.00	0.98	44.24
3	Maintenance	7.65	31.63	2.42	28.69	2.19	30.00	2.30	44.24
1	Supermaintenance	16.00	22.00	3.52	65.26	10.44	2.00	0.32	64.35
2	Supermaintenance	14.22	24.75	3.52	54.92	7.81	10.00	1.42	64.35
3	Supermaintenance	11.12	31.63	3.52	28.69	3.19	30.00	3.34	64.35

whereas the rats on the 10%-fat and the 30%-fat diets received smaller quantities of food which contained the same quantities of gross energy and of protein as supplied by the 2%-fat diet.

The schedule of experimentation, given in table 2, was so arranged for each rat that the 2-day maintenance respiration period began immediately after the 8-day maintenance excreta collection period. Then followed (a) 5½ days' preparatory feeding on the supermaintenance plane, (b) the 2-day respiration period on supermaintenance feeding, and (c) the 8-day excreta collection period on supermaintenance feeding — the object of this arrangement being to have the respiration

periods at the two planes of nutrition as close together, in point of time, as practicable.

The digestibility of the food nitrogen (table 3) was essentially the same at both planes of food intake, such difference as existed being an increase in the order of the increase in

TABLE 2

Schedule of experimentation.

RAT NO.	PLANE OF NUTRITION	FAT CONTENT OF DIET	DAILY ENERGY INTAKE	AGE OF RATS	AVE. WEIGHT OF RATS	DATES OF RESPIRATION MEASUREMENTS
		%	*Cal.*	*days*	*gm*	
1–12	Maintenance	2	44.24	169	311	Jan. 28–Feb. 2
1–12	Supermaintenance	2	64.35	176	335	Feb. 4–Feb. 9
13–24	Maintenance	10	44.24	169	310	Mar. 4–Mar. 9
13–24	Supermaintenance	10	64.35	176	335	Mar. 11–Mar. 16
25–36	Maintenance	30	44.24	169	326	Apr. 1–Apr. 6
25–36	Supermaintenance	30	64.35	176	350	Apr. 8–Apr. 13

TABLE 3

Utilization of daily nitrogen.

DIET NO.	PLANE OF NUTRITION	FAT CONTENT OF DIET	NITROGEN INTAKE	NITROGEN DIGESTED		NITROGEN OF URINE		NITROGEN RETENTION	
		%	*mg*	*mg*	*% of intake*	*mg*	*% of intake*	*mg*	*% of intake*
1	Maintenance	2	387	356	92.0	360	93.0	— 4	— 1.0
2	Maintenance	10	387	358	92.5	356	92.0	2	0.5
3	Maintenance	30	387	360	93.0	375	96.9	— 15	— 3.9
1	Supermaintenance	2	563	518	92.0	445	79.0	73	13.0
2	Supermaintenance	10	563	522	92.7	457	81.2	65	11.5
3	Supermaintenance	30	563	522	92.7	475	84.4	47	8.3

the fat content of the diets. At the higher plane of food intake the nitrogen retention decreased in the order of the increasing fat content of the diets, the odds that the retention from the 2%-fat diet was greater than from the 30%-fat diet being more than 10,000 to 1. This fact is not explained by any of the experimental observations made; but in view of the proba-

bility that the composition of the diets affected the synthetic activities of the alimentary microörganisms, it is not surprising that the reasons for some of the facts observed are not in evidence.

Referring to table 4 — at both planes of energy intake there were, in the order of the increasing fat content of the diets, barely perceptible increases in the energy of the daily urine; definite increases in the fecal energy; definite decreases

TABLE 4

Intake and partition of daily food energy.

DIET NO.	PLANE OF NUTRITION	INTAKE	FECES	URINE	METABOLIZABLE ENERGY		HEAT PRODUCTION		HEAT PRODUCTION DIVIDED BY METABOLIZABLE ENERGY
		Cal.	*Cal.*	*Cal.*	*Cal.*	*Coef. var. %*	*Cal.*	*Coef. var. %*	
1	Maintenance	44.24	1.99	3.07	39.18	0.31	30.37	6.92	0.78
2	Maintenance	44.24	2.27	3.08	38.89	0.59	29.33	5.96	0.75
3	Maintenance	44.24	2.99	3.11	38.14	0.84	27.93	5.19	0.73
1	Super-maintenance	64.35	3.82	4.37	57.16	0.41	36.53	2.80	0.64
2	Super-maintenance	64.35	3.32	4.41	56.62	0.46	34.52	5.14	0.61
3	Super-maintenance	64.35	4.57	4.43	55.35	1.00	31.22	3.03	0.56

in the metabolizable energy; and decreases in the heat production which were definitely more extensive than the decreases in metabolizable energy.

The values for heat production divided by metabolizable energy at the lower plane of nutrition were sufficiently less than unity to indicate that the energy intake at this lower plane of nutrition was well above the maintenance requirement including normal activity. That the values for heat production divided by metabolizable energy diminished in the order of the increase in per cent of fat in the diets signifies that the fat component of the diets conferred efficiency of utilization of their metabolizable energy.

At the higher plane of nutrition the computed odds that the metabolizable energy of the 10%-fat diet was more efficiently utilized than that of the 2%-fat diet were 131 to 1; and the odds that the metabolizable energy of the 30%-fat diet was more efficiently utilized than that of the 2%-fat diet and of the 10%-fat diet were millions to one.

The quantities of heat, and the differences in the amounts of heat, as well as the sources of the heat produced from each diet, are given in table 5. The total heat production at both

TABLE 5

Quantities, increments, and sources of average daily heat production.

DIET NO.	PLANE OF NUTRITION	TOTAL HEAT AND HEAT INCREMENTS	NON-PROTEIN R.Q.	SOURCES OF HEAT PRODUCTION			
				Protein	Carbohydrate	Fat	Fat Synthesis
		Cal.		*Cal.*	*Cal.*	*Cal.*	*Cal.*
1	Supermaintenance	36.53	1.20	11.80	23.70	0.00	1.03
1	Maintenance	30.37	1.10	9.54	20.43	0.00	0.40
1	Heat increment	6.16		2.26	3.27		0.63
2	Supermaintenance	34.52	1.09	12.12	21.98	0.00	0.42
2	Maintenance	29.33	.97	9.44	18.00	1.89	0.00
2	Heat increment	5.19		2.68	3.98		0.43
3	Supermaintenance	31.22	.85	12.59	9.45	9.18	0.00
3	Maintenance	27.93	.80	9.93	6.01	11.99	0.00
3	Heat increment	3.29		2.66	3.43		0.00

planes of nutrition, as well as the heat increment for each diet, diminished markedly in the order of the increasing fat contents of the diets.

The conditions of experimentation determined that the heat increments represented the efficiency of utilization of food at a plane of nutrition above maintenance, with voluntary activity excluded, by animals nearing maturity but still able to store considerable amounts of nutriment. The proportions of the heat increments derived from carbohydrate and protein from each diet were similar, but not identical, and these values

appear not to have been affected by the diets in regular order.

With reference to the sources of the total heat production — the greatest difference observed was the decrease in heat from carbohydrate at both planes of nutrition, in the order of the increasing fat content of the diets. Similarly, there was a slight increase in heat production from protein, with one departure from the regular order. No fat was catabolized from the 2%-fat diet, while much heat was produced from fat from the 30%-fat diet. Heat production from fat synthesis occurred in the three periods in which the carbohydrate intake was highest, and ranged from 1.3 to 2.8% of the total heat production.

The results of this experiment, in which the intake of the vitamins was purposely excessive, were in general agreement with the earlier experiment, in which the vitamin intake was not so large, especially in that the heat production at both planes of food intake, and also the heat increment of the diets, decreased in the order of their increasing fat contents.

These findings, together with the demonstration by Forbes, Swift, Marcy and Davenport ('44) that the dynamic effects of isocaloric diets decrease in the order of their increasing protein contents, seem harmonious with the fact that protein and fat are the main components of the animal body.

SUMMARY

Respiration experiments were conducted by the open-circuit Haldane respiratory quotient procedure, with thirty-six mature albino rats as subjects, to investigate the energy expense of utilization (heat increment) of three complete diets differing in fat content and supplying ten vitamins in much larger quantities than in earlier experiments.

The heat increments were measured as the difference in heat production from maintenance and supermaintenance diets containing 2, 10 and 30% of fat, respectively, so compounded and fed as to supply equal quantities of gross energy, protein, and vitamins.

The fat content of the diets had little effect on nitrogen utilization, but with the supermaintenance food intake there was slight increase in urinary nitrogen and decrease in nitrogen retention in the order of the increasing fat content of the diets. There were slight decreases in metabolizable energy and larger decreases in heat production in the order of the increasing fat contents of the diets, the fat component, therefore, conferring economy of utilization of food energy.

The heat increments of the dietary supplements containing 2, 10 and 30% of fat, respectively, representing the use of food above maintenance, with voluntary activity excluded, were equivalent to 31, 26 and 16%, respectively, of their gross energy.

The decreasing energy expense of utilization of the isocaloric intake of the diets in the order of their increasing fat contents was due mainly to decreasing heat from the catabolism of carbohydrates. No fat was catabolized from the 2%-fat diet, while much heat was produced from fat in the 30%-fat diet.

Heat production from fat synthesis occurred in the three periods in which the carbohydrate intake was highest.

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DENTAL CARIES IN THE COTTON RAT

VIII. FURTHER STUDIES ON THE DIETARY EFFECTS OF CARBOHYDRATE, PROTEIN AND FAT ON THE INCIDENCE AND EXTENT OF CARIOUS LESIONS [1]

B. S. SCHWEIGERT, ELIZABETH POTTS, JAMES H. SHAW,[2] MARIE ZEPPLIN AND P. H. PHILLIPS

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

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Previous studies from this laboratory (Shaw et al., '44 and Schweigert et al., '45b, '46) have shown that the incidence and extent of tooth decay in the cotton rat were markedly influenced by the kind of carbohydrate in the diet. Specifically, ingestion of sucrose or other soluble sugars resulted in a high caries index, while few carious lesions developed on dextrin or stock rations. With diets in which lard or casein had been isocalorically substituted for sucrose, a low caries index was found, and animals fed milk as the sole diet were free of carious lesions (Schweigert et al., '46). It was therefore of interest to study the effects of carbohydrates other than sucrose in diets containing added lard or increased amounts of protein.

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We are indebted to Merck and Company, Rahway, New Jersey, for the crystalline vitamins; to Abbott Laboratories, Chicago, for the halibut liver oil; and to Wilson and Company, Chicago, for the 1 : 20 liver extract and dog food used in this study.

[2] Now at the Harvard School of Dental Medicine.

EXPERIMENTAL

Weanling cotton rats (15–25 gm) from our stock colony were used in these experiments. The animals were kept on experiment for 14 weeks (Shaw et al., '44) and the growth rate in grams per week was recorded as the growth of males as pointed out by Schweigert et al. ('45b). Since a strain variation in caries susceptibility has been observed (Schweigert et al., '45a) animals from each litter were equally distributed among all experimental groups and the control group fed the sucrose basal ration. At the end of the experimental period, the animals were sacrificed and the incidence and extent of the carious lesions were determined by the method of Shaw et al. ('44).

The rations were prepared at weekly intervals and fed ad libitum. The composition of the rations is shown in table 1.

TABLE 1

Composition of rations.[1]

CONSTITUENT (PARTS OF THE RATION)	SUCROSE	LACTOSE	DEXTRIN	STARCH	GLUCOSE	DEXTRI-MALTOSE	CASEIN	COEN OIL	SALTS IV	LARD
Ration no.										
802	67	....	....	..	...	....	24	5	4	..
817	....	....	....	67	...	....	24	5	4	..
818	....	....	67	..	...	....	24	5	4	..
819	....	33.5	33.5	..	...	....	24	5	4	..
833	....	16.75	50.25	..	...	....	24	5	4	..
825	16.75	....	50.25	..	...	....	24	5	4	..
821	44.5	....	....	..	...	....	24	5	4	10
826	....	44.5	....	..	...	....	24	5	4	10
827	22.25	22.25	....	..	...	....	24	5	4	10
828	....	....	....	..	...	44.5	24	5	4	10
829	....	....	....	..	44.5	....	24	5	4	10
830	22.25	..	....	..	...	22.25	24	5	4	10
831	32.5	....	....	..	...	....	36	5	4	10
832	22.25	.. .	22.25	..	...	....	24	5	4	10

[1] Adequate quantities of the B vitamins were provided (McIntire, Schweigert and Elvehjem, '44).

When lard or additional protein was included in the ration, 1 gm of the former was added at the expense of 2.25 gm of carbohydrate, and 1 gm of protein replaced 1 gm of the carbohydrate.[3] Adequate quantities of the B vitamins were provided (McIntire et al., '44) and each rat received 1 drop of halibut liver oil per week. Four per cent of 1 : 20 liver extract was added to each diet at the expense of the entire ration.

RESULTS AND DISCUSSION

The growth rates of all animals for the first 6 weeks and the total 14 weeks on experiment are shown in tables 2 and 3.

TABLE 2

Effects of various carbohydrates on the incidence and extent of carious lesions.[1]

RATION FED[2]	NO. OF ANIMALS	RATE OF GROWTH		AVE. INCIDENCE OF CARIOUS LESIONS	AVE. EXTENT OF CARIOUS LESIONS
		First 6 weeks	Total 14 weeks		
		gm/week			
802	7	10.2	8.2	23	70 +
817 (starch)	6	6.6	4.6	2	7 +
802	7	9.4	6.5	32	97 +
819 (½ dextrin, ½ lactose)	7	10.1	6.5	23	63 +
818 (dextrin)	3	9.8	7.0	1	2 +
802	6	10.6	7.6	33	94 +
833 (¾ dextrin, ¼ lactose)	4	8.8	7.0	18	46 +
802	4	13.1	8.6	25	57 +
825 (¾ dextrin, ¼ sucrose)	4	12.3	7.8	13	19 +

[1] For a detailed description of the method of evaluating the incidence and extent of the lesion see Shaw et al. ('44).

[2] 4% of 1 : 20 liver extract was added to all diets at the expense of the entire ration. The proportions of the carbohydrate portion of the diet are given in parentheses.

The ingestion of rations in which starch or a combination of lactose and lard had been substituted for sucrose (rations 817 and 826, respectively) resulted in a somewhat slower rate of growth, while the growth rates obtained with the other

[3] Glucose was fed as cerelose, a commercial glucose monohydrate. The water of crystallization was taken into account in preparing the rations.

rations were not appreciably different from those of animals fed the sucrose basal. In previous work from this laboratory high lactose diets were found to promote slower growth (Schweigert et al., '45b).

TABLE 3

Effects of various carbohydrates and of the level of fat, carbohydrate, and protein on the incidence and extent of carious lesions.

RATION FED [1]	NO. OF ANIMALS	RATE OF GROWTH		AVE. INCIDENCE OF CARIOUS LESIONS	AVE. EXTENT OF CARIOUS LESIONS
		First 6 weeks	Total 14 weeks		
		gm/week			
802 (sucrose basal)	10	11.0	8.3	28	74 +
821 (sucrose, lard)	7	11.9	8.3	17	42 +
827 (½ sucrose, ½ lactose, lard)	5	9.1	6.7	14	26 +
826 (lactose, lard)	1 [2]	6.8	3.0	10	24 +
802 (sucrose basal)	3	10.3	7.2	22	67 +
828 (dextri-maltose, lard)	4	12.3	8.4	7	12 +
829 (glucose, lard)	4	9.5	7.1	9	16 +
802 (sucrose basal)	5	10.2	6.8	35	101 +
821 (sucrose, lard)	5	10.5	6.6	16	32 +
828 (dextri-maltose, lard)	3	9.2	6.5	26	55 +
829 (glucose, lard)	4	9.0	5.6	24	47 +
802 (sucrose basal)	6	11.4	8.2	31	82 +
821 (sucrose, lard)	3	13.3	10.4	15	32 +
830 (½ sucrose, ½ dextri-maltose, lard)	7	11.8	8.3	18	34 +
832 (½ sucrose, ½ dextrin, lard)	5	11.8	8.7	17	30 +
831 (sucrose, added casein, lard)	5	11.5	8.0	9	17 +

[1] 4% of 1 : 20 liver extract was added to each diet at the expense of the entire ration. The proportions of the carbohydrate and the presence of 10 parts of lard are indicated in the parentheses.

[2] 3 of the 4 animals in this group failed to survive the 14-week experimental period.

The average incidence and extent of the carious lesions for each of the groups are shown in tables 2 and 3. The results obtained from the initial and repeat experiments were averaged together unless the susceptibility of the control groups

was different. In the latter case the results from each experiment are presented.

The replacement of sucrose by starch resulted in a marked decrease in the severity of tooth damage, the averages for incidence and extent of lesions being 23 and 70+ and 2 and 7+, respectively. The latter index is comparable to that obtained with dextrin or stock rations. Among the carbohydrates tested as the sole carbohydrate in ration 802, starch and dextrin are the only ones that have afforded protection, as opposed to sucrose, glucose, dextri-maltose, fructose, maltose, or lactose. Honey was fed to one group of animals as the source of carbohydrate (by replacement of the sucrose in ration 802) and some protection was noted.

Animals fed a diet of whole liquid milk have been found to be free of dental caries. Therefore, experiments were conducted to obtain further information about the effect of lactose on caries development. Since a high mortality occurs when lactose diets are fed, rations containing less than 50 parts of lactose were prepared for the purpose of contrasting sucrose and lactose. In earlier work (Schweigert et al., '45b) rations containing ½ dextrin and ½ sucrose as the carbohydrate portion gave results comparable to those with the sucrose basal. In the present work a slight protection was observed with ½ dextrin and ½ lactose. However, the results of ¾ dextrin — ¼ sucrose and ¾ dextrin — ¼ lactose were similar (table 2). Other experiments were conducted with diets in which 10 parts of lard were added at the expense of the carbohydrate. The ingestion of such diets with sucrose as the carbohydrate has resulted in partial protection and the positive or negative effect of lactose could be studied. The results obtained with rations 826 and 827 indicated that lactose gave somewhat better protection against caries in these lard rations than did sucrose. Unfortunately, however, only one animal survived the experimental period when ration 826 (44.5 parts of lactose) was ingested (table 3). The interrelationship of the kind and level of carbohydrate and fat may be more clearly

demonstrated when other fats are tested in diets similar to these. Such experiments are in progress.

In order to obtain further information about the effects of added protein as well as of other carbohydrates, namely glucose, dextri-maltose, and dextrin, these constituents were added at the expense of the remaining sucrose in the rations containing 10 parts of lard. On the low fat, high carbohydrate diets, a high caries experience occurred with glucose and dextri-maltose as well as with sucrose. In the rations containing 10 parts of lard, glucose and dextri-maltose resulted in caries indices at least as high as those with sucrose (table 3). Similarly, severe lesions were observed when mixtures of sucrose and dextri-maltose or sucrose and dextrin comprised the carbohydrate portion of the diet (rations 830 and 832, respectively). The latter result is somewhat surprising since dextrin has been found to give a low caries index. In earlier work, a low caries occurrence was found with either coarse or fine dextrin rations. Consequently, no attempt was made in the present work to control the particle size of the dextrin used. It is possible that in combination with sucrose the particle size of the dextrin may exert an influence on the initiation of carious lesions in the cotton rat. Ingestion of any of the diets containing lard, however, gave partial protection as compared with the results with animals fed the low fat, sucrose basal ration. Increasing the protein (from 24 to 36 parts) in addition to raising the fat level (ration 831) lowered the caries index from 15 and 32+ to 9 and 17+. Thus, an additive effect of the increased protein and added lard was demonstrated. In earlier work, the effects of the protein and lard were also found to be additive, 50 parts of casein and 10 parts of lard resulting in practically complete protection (1 carious lesion per rat).

These studies confirm earlier observations in that the addition of dextrin, lard, or additional protein at the expense of the sucrose markedly reduced the caries occurrence. Glucose or dextri-maltose as the sole carbohydrate in a low fat diet has been found to be comparable to sucrose in the effect on

dental caries incidence and extent. Similarly, animals fed sucrose, glucose, or dextri-maltose in rations containing 10 parts of lard had a comparable caries occurrence. The effect of lactose appeared to be intermediate between those noted for sucrose and dextrin, although the data are as yet too meager to warrant definite conclusions.

In other experiments the effect of feeding dextrin or sucrose for 14 weeks was compared with that found by feeding dextrin for 4 or 6 weeks and then the sucrose basal ration for 14 weeks. The incidence and extent for the sucrose controls were 27 and 72+ and for the dextrin controls 1 and 2+. However, those that received dextrin rations for 4 or 6 weeks prior to receiving the sucrose ration had a high caries index similar to that of those fed the sucrose ration from weaning. It appears, therefore, that although the animals were older, they were as susceptible to caries as weanlings when fed the sucrose ration for a comparable period. Another series composed of 3 groups was fed the sucrose basal for 14 weeks; the dextrin basal for 10 weeks, then the sucrose basal for 4; and the dextrin basal for 8 weeks, then the sucrose basal for 6. The averages for caries incidence and extent were 23 and 67+; 0; and 10 and 21+, respectively. Feeding the sucrose ration for 4 or 6 weeks was therefore insufficient for maximum caries development.

The average caries index for the offspring from each stock pair fed the sucrose control ration has been computed for approximately 100 animals representing 20 stock pairs during the past year. In general the average susceptibility of the offspring for the entire colony has increased slightly as compared with that of siblings of the parents (Schweigert et al., '45a). The animals obtained from Michigan and Florida have a slightly lower average incidence and extent than the Wisconsin stock. In general the caries susceptibility of the offspring tends to parallel that of the parent stock. No differences in the caries experience of offspring of the stock pairs could be attributed to the number of litters born, the time between litters, or the number of animals in each litter.

SUMMARY

1. The ingestion of diets in which starch or dextrin had been substituted for sucrose resulted in a low incidence and extent of dental caries in the cotton rat as compared with those found after ingestion of the control sucrose ration.

2. Diets in which 10 parts of lard had been added at the expense of the carbohydrate gave some protection as compared with the low fat, sucrose ration. No difference was observed in the effects of glucose, dextri-maltose, or sucrose in these lard rations.

3. With sucrose rations containing 10 parts of lard, increasing the protein level at the expense of the remaining carbohydrate further reduced the caries occurrence.

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THE UTILIZATION OF CALCIUM IN SOYBEAN PRODUCTS AND OTHER CALCIUM SOURCES[1]

LAWRENCE J. SCHROEDER, WILLIAM M. CAHILL AND ARTHUR H. SMITH

Department of Physiological Chemistry, Wayne University College of Medicine, Detroit, Michigan

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Data have been presented previously (Cahill, Schroeder and Smith, '44) indicating the magnitude of the soybean crop in this country and the portion which could be diverted into channels for human consumption. Although the soybean is grown largely for its oil, of which it contains about 20%, the protein, comprising some 34% of the seed has been shown to be of high quality in human nutrition. In addition, the dry seed is a comparatively rich source of calcium among plant products (0.20%). Inasmuch as this legume promises to become more important in our national dietary, an estimate of the availability of its various nutrients in man is important.

Studies of the utilization by man of the calcium in plant sources indicate that in general, the calcium in most green leaves (lettuce — Mallon, Johnson and Darby, '33; spinach — Bonner, Hummel, Bates, Horton, Hunscher and Macy, '38; greens — Speirs, '39) compares favorably with that in milk in this respect, whereas the retention from roots (taro — Potgieter, '40; carrots — Breiter, Mills, Rutherford, Armstrong and Outhouse, '42) is somewhat poorer. As regards the availability of the calcium in legume seeds, Pittman ('32) observed that with 5.5 to 6.5 mg of calcium per kg body weight, 80–85% of which was furnished by cooked navy beans, there

[1] Supported by a grant from the Nutrition Foundation, Inc.

was a persistent negative calcium balance in five women subjects. No attempt was made to compare the retention of calcium when it was provided from different sources. In a study of utilization of calcium by three Chinese subjects, Adolph and Chen ('32) fed 8.1 mg per kg body weight of calcium, 80% of which was from cow's milk in control periods and from soybean curd, in experimental periods. On the basis of the calcium balance under these conditions, they concluded that soybean curd and bovine milk are equally effective as sources of calcium in the Chinese diet.

The present report contains data on the efficiency of utilization by adult human subjects of calcium in soybean "milk," in whole cooked soybeans, in evaporated milk, and in calcium sulfate.[2]

EXPERIMENTAL

The experimental subjects were thirteen male medical students. Data on the ages, body weights, calorie intake, etc., are given in table 2.

Prior to the beginning of the experimental period, the energy requirement was calculated for each subject and proper adjustment then made during the first days of the preliminary period to insure a subjective sense of adequacy of intake. After an initial period of 10 days, the basal experimental period of 20 days followed. The 20-day periods were divided into 5-day groups for analytical purposes and only the last 3 of the 5-day periods were used in making the final calculations in order to avoid the influence of a metabolic lag in the experimental subjects. A total of forty-nine balance periods, each 20 days in length was the source of the data herein presented. Owing to limitation of facilities, there were three separate diet squads of six men each, one squad being studied at a time. There was no indication that the

[2] Shortly after this study was begun, the Civilian Food Requirements Board of the Food Distribution Administration requested data regarding the availability of the calcium from calcium sulfate. This topic was thus made part of the present investigation.

dietary regime affected the health of the subjects adversely and the changes in body weight were insignificant.

The philosophic approach developed by Steggerda and Mitchell ('39) was used in the evaluation of the efficiency of utilization of calcium in the present study. A negative balance was induced by a calcium-poor ration, in the present case by the preliminary 10-day period plus the first basal 20-day period. The basal period was followed, in most cases, by two 20-day periods in which the basal diet was supplemented with calcium, in the form of a pure salt or as a food. The amount of the calcium supplement was estimated by subtracting the calcium consumed in the basal diet from the total requirement (Steggerda and Mitchell, '41) calculated to the body weight of the subject (9.55 mg per kg body weight). The extent of utilization of the calcium in the tested supplement is computed by equating the spared calcium with the supplemental calcium. In all cases in which the supplement was a food, adjustments in the basal diet were made so as to keep constant the energy value and the protein content of the preliminary low-calcium diet and of the experimental supplemented ration. An adequate protein level was provided (see Kunerth and Pittman, '39; Pittman and Kunerth, '39). Nitrogen balance studies were run on the subjects of Diet Squad I to insure this adequacy of protein level; all members were found to be in nitrogen balance or very nearly so. Three of the diets eaten by one of the experimental subjects are shown in table 1.

The basal diet included bacon, tomato juice, white bread, coffee, oatmeal, apples, cookies, ground beef, canned corn, potatoes, canned applesauce, oleomargarine, and sucrose. The canned foods were of one brand and amounts sufficient for the entire experiment were purchased at one time. The meat was purchased in large quantities, made into 60 gm patties, wrapped, and frozen. Apples and potatoes were purchased as frequently as limited storage facilities required. Foods were sampled periodically and calcium determinations made on aliquots of pooled samples. The supplements used as main

TABLE 1

Sample diets (J.H., 69.1 kg, Diet Squad I).

FOODSTUFF [1]	BASAL DIET				$CaSO_4 \cdot 2H_2O$ SUPPLEMENTED DIET				EVAPORATED MILK SUPPLEMENTED DIET			
	Food		Protein	Calcium [11]	Food		Protein	Calcium [11]	Food		Protein	Calcium
	gm	*Cal.*	*gm*	*mg*	*gm*	*Cal.*	*gm*	*mg*	*gm*	*Cal.*	*gm*	*mg*
Bacon [2]	90	540	11.0	12.2	90	540	11.0	12.2	90	540	11.0	12.2
Bread	50	130	4.8	35.6	50	130	4.8	35.6	50	130	4.8	35.6
Oatmeal [3]	100	391	14.2	47.4	100	391	14.2	47.4	100	391	14.2	47.4
Apple [4]	300	180	0.9	26.1	300	180	0.9	26.1	300	180	0.9	26.1
Cookies [5]	60	260	6.0	16.5	60	260	6.0	16.5	60	260	6.0	16.5
Beef [6]	120	280	22.4	13.1	120	280	22.4	13.1	58	135	10.8	6.3
Corn [7]	150	142	3.8	8.6	150	142	3.8	8.6	150	142	3.8	8.6
Potato [8]	250	200	5.0	27.6	250	200	5.0	27.6	250	200	5.0	27.6
Applesauce	200	160	0.4	7.2	200	160	0.4	7.2	200	160	0.4	7.2
Tomato juice	200	44	2.0	19.2	200	44	2.0	19.2	200	44	2.0	19.2
Oleomargarine	60	440	0.1	11.3	60	440	0.1	11.3	58	425	0.1	11.3
Coffee [9]	360	...	...	13.6	360	...	..	13.6	360	...	..	13.6
Sucrose	55	220	0.0	0.0	55	220	0.0	0.0	38	152	0.0	0.0
Vitamin supplement	2 capsules	...	...	1.2	2 capsules	...	..	1.2	2 capsules	...	..	1.2
$CaSO_4 \cdot 2H_2O$ [10]	...	...	...	...	1.79	0.0	0.0	415.0	...	...	..	...
Evaporated milk	...	...	...	...		...	..	....	166	231	11.6	406.1
Totals		2987	70.6	239.6		2987	70.6	654.6		2990	70.6	638.1
Protein calories												
Total			282.4				282.4				282.4	
Per cent			9.43				9.43				9.43	
Calcium distribution												
Basal food			100%				36.6%				36.4%	
Supplement			0%				63.4%				63.6%	

[1] Distilled water was allowed the subjects ad libitum.

[2] The bacon was fried such that the weight-loss represented one-fourth of the original wet-weight. The loss fat was taken into account in computing food calories. The fat extract was analyzed and no detectable calci was found.

[3] Based on dry-weight of the oatmeal. The food was cooked with water in the usual manner.

[4] The apples were cored and quartered before eating.

[5] The cookies, made from a milk-free batter, were obtained from a local wholesale bakery.

[6] The beef patties were fried in a minimum of bacon fat.

[7] Drained, canned corn; heated before serving.

[8] Based on wet-weight of the scrubbed, washed potatoes. They were oven-baked before serving and consum entirely (peel included).

[9] The coffee was prepared by percolation with distilled water. One level teaspoonful of coffee was used p cup; analyses for calcium were based on this infusion.

[10] Calcium supplements were divided into three equal portions daily and consumed at mealtime.

[11] Average calcium values of various pooled food samples during each diet were used in computing food-calci percentages.

sources of calcium in the experimental periods were evaporated milk,[3] soybean "milk,"[4] whole soybeans[5] and calcium sulfate.[6]

The calcium sulfate, evaporated milk and soybean "milk" supplements each provided all of the added calcium in the appropriate period and were added three times a day at mealtime. However, in the periods where whole soybeans were fed, only half of the added calcium was secured from this source because of the difficulty in consuming the requisite large amounts of this vegetable; the remaining supplementary calcium in these periods was given as calcium sulfate. A capsule containing 1.5 mg of thiamine chloride, 2.0 mg of riboflavin, 1.0 mg of calcium pantothenate, 10 mg of niacinamide, 0.10 mg of pyridoxine and yeast and liver extract, as well as another containing 5000 units of vitamin A and 500 units of vitamin D, were consumed each day by each subject.[7]

The soybeans used as a calcium supplement were prepared by autoclaving the whole beans with two parts of water at 15 pounds pressure for 1 hour. The soybean "milk" was usually diluted with water and chilled before serving. The preparation of the foods of the basal diet may be seen in the footnotes of table 1.

All analyses for calcium were made by a modification of the method of Shohl and Pedley ('22). Calcium-free charcoal markers were used to separate the feces into 5-day periods; the fresh feces were covered with 95% ethanol and dried on steam cones after which analyses were made on aliquots of the dried, ground material. Both food and fecal analyses

[3] Whitehouse brand, not irradiated.

[4] Mull-soy, provided by the Borden Company. Mull-soy is an emulsified concentrate of water, soybean flour, soybean oil, dextrose, sucrose, calcium phosphate, calcium carbonate, salt and soybean lecithin; homogenized and sterilized. Its soybean proteins are practically the nutritional equivalent of egg proteins (Cahill, Schroeder and Smith, '44).

[5] Run-of-the-mill field-grown soybeans were used rather than a single variety. The soybeans were obtained from the Glidden Company, through the courtesy of Dr. Percy Julian.

[6] $CaSO_4 \cdot 2\,H_2O$, Merck's Reagent.

[7] Kindly provided by the Gelatin Products Company.

were carried out on samples previously "flamed" and ashed at 550–600°C. Twenty-four-hour urine specimens were collected daily, and an aliquot preserved with hydrochloric acid and toluene. After a 5-day collection period, the pooled urine samples from one subject were again adjusted to volume and aliquots removed for analysis. Preliminary oxidation with ammonium persulfate and nitric acid were made prior to the calcium determinations on the urine samples. The calcium oxalate precipitates were filtered through 10 mm Pyrex micro filter sticks (medium porosity) to exclude calcium losses through ordinary filtration. The precipitates were washed with 1:50 NH_4OH before dissolving in hot normal sulfuric acid and subsequent titration with potassium permanganate.

DISCUSSION OF RESULTS

The level of intake of calcium in the periods when only the basal diet was consumed varies from 153 to 276 mg daily, depending on the energy requirements of the subject. During these basal periods the subjects showed a negative calcium balance (see table 2). Using the value of 9.55 mg per kg body weight per diem (Steggerda and Mitchell, '41) as the calcium requirement of the adult, the total daily need was found; from this value, the daily basal intake was subtracted, the result being the amount of calcium to be added in the form of the supplement under investigation. This procedure should have brought the subject into calcium balance; actually, owing to a lag in securing final analytical data, the subjects were usually in slight negative balance after the supplementation. The decrease in the amount of endogenous calcium loss brought about by the added calcium in the supplement, divided by the added supplementary calcium is the index of availability or utilization of the added calcium.

In general on the basal diet, close to two-thirds of the calcium was excreted in the feces; when calcium sulfate was added, total excretion of calcium was increased but the partition between urine and feces remained unchanged. On the other hand, when evaporated milk or soybean "milk" was

added, the increment in loss was largely by way of the gut. It thus appears that whatever the significance of fecal calcium, the calcium from calcium sulfate was well absorbed under the present experimental conditions and is excreted in greater amounts by the kidney than is the calcium from the other supplements used.

As seen in table 2, the addition of supplementary calcium in the form of $CaSO_4 \cdot 2\,H_2O$, evaporated milk or soybean "milk," prevents a considerable amount of endogenous calcium loss. It should be pointed out that the evaporated milk was not irradiated and that the soybean "milk," made from a low-fat soybean flour, had been fortified with calcium phosphate. However, adequate vitamin D was given the subjects as a daily supplement to the basal diet.

A total of eleven subjects were used in the study of the utilization of calcium in hydrated calcium sulfate and the final calculations show that under the present experimental conditions the average per cent utilization was 23.7. The values as may be seen in table 2 ranged from 7.2 to 34.6%. With a comparable number of subjects the calcium utilization with soybean "milk" was 22.6% with a range of from 9.9 to 56.1. These values compare favorably with those obtained in the evaporated milk studies. In the latter instance a limited number of subjects (6) was used and the values obtained ranged from 13.7 to 41.8% with an average utilization of 22.6. Only four of the subjects completed the experiment on whole soybeans; however, from the limited data (average per cent utilization was 10.4 and the range was from 2.2 to 18.1) it would appear that not only is the cooked whole soybean not a good source of dietary calcium, but also, when fed in large quantities, it interferes with the utilization of other dietary calcium. A similar action has been observed by Morgan ('34) in human subjects in connection with regenerated cellulose. Inasmuch as little such interference is noted with the soybean "milk," it seems likely that the difficulty may reside in the relatively large amount of roughage in the whole soybeans.

TABLE 2

Utilization of calcium in soybeans and other calcium sources based on the daily average metabolism of calcium.

SQUAD AND DATE	SUBJECT AND AGE YEARS	BODY WEIGHT		CALCIUM SUPPLEMENT TESTED	TEST PERIOD DAYS [1]	AVERAGE DAILY METABOLISM OF CALCIUM					BALANCE
		Beginning	Weight change			Total calorie intake	Calcium intake		Calcium excretion		
							Basal diet	Supplement	Feces	Urine	
		kg	*kg*				*mg*	*mg*	*mg*	*mg*	*mg*
Diet Squad I October 12 to December 11, 1943	R.C. 23	85.4	−0.91	None	20	3697	273	0	302	158	−187
				$CaSO_4 \cdot 2H_2O$	20	3697	273	540	653	248	− 88
				Evaporated milk	20	3683	264	530	688	171	− 65
	W.C. 23	76.4	+0.91	None	20	3462	276	0	272	136	−132
				$CaSO_4 \cdot 2H_2O$	20	3462	276	445	516	220	− 15
				Evaporated milk	20	3474	269	436	638	140	− 73
	F.G. 22	62.1	−0.23	None	20	2987	226	0	189	245	−208
				$CaSO_4 \cdot 2H_2O$	20	2987	226	367	372	302	− 81
				Evaporated milk	20	2986	220	360	422	197	− 39
	J.H. 23	69.1	+0.91	None	20	2987	240	0	265	156	−181
				$CaSO_4 \cdot 2H_2O$	20	2987	240	415	489	236	− 70
				Evaporated milk	20	2990	233	406	516	169	− 48
	P.T. 26	58.4	−0.45	None	20	2792	216	0	242	94	−120
				$CaSO_4 \cdot 2H_2O$	20	2792	216	324	454	129	− 43
				Evaporated milk	20	2791	210	319	510	95	− 76
	S.Z. 24	63.6	−0.45	None	20	2792	215	0	219	188	−192
				$CaSO_4 \cdot 2H_2O$	20	2792	215	380	475	199	− 79
				Evaporated milk	20	2789	209	372	507	113	− 39
Diet Squad II April 18 to June 22, 1944	F.C. 21	72.6	+0.91	None	20	3547	237	0	260	112	−135
				$CaSO_4 \cdot 2H_2O$	20	3547	237	440	545	159	− 27
				Soy milk	20	3556	227	490	629	131	− 43
	C.C. 21	59.9	+0.23	None	20	3088	203	0	222	69	− 88
				$CaSO_4 \cdot 2H_2O$	20	3088	203	354	461	83	+ 13
				Soy milk	20	3085	189	394	410	48	+125
	R.K. 22	71.3	+0.45	None	20	3367	233	0	233	203	−203
				$CaSO_4 \cdot 2H_2O$	20	3367	233	431	562	274	−172
				Soy milk	20	3372	233	481	608	197	− 91
	M.K. 20	69.0	−0.91	None	20	3160	199	0	231	46	− 78
				$CaSO_4 \cdot 2H_2O$	20	3160	199	448	533	68	+ 46
				Soy milk	20	3155	190	498	631	58	− 1
	P.T. 26	61.7	0.0	None	20 [2]	3167	202	0	194	79	− 71
				$CaSO_4 \cdot 2H_2O$	20	3167	202	369	443	151	− 23
				Soy milk	20	3143	194	413	499	139	− 31
	S.Z. 24	63.5	−0.23	None	20	2932	197	0	204	107	−114
				Soy milk	20	2927	189	592	596	109	+ 76
Diet Squad III July 13 to September 22, 1944	R.A. 26	100.9	−1.82	None	20	3775	219	0	236	256	−273
				Soy milk	20	3775	194	795	831	261	−103
				Soybeans and $CaSO_4 \cdot 2H_2O$	20	3770	182	715 [3]	904	251	−258
	R.I. 27	55.4	+0.91	None	20	2266	171	0	206	98	−133
				Soy milk	20	2275	166	389	536	87	− 69
				Soybeans and $CaSO_4 \cdot 2H_2O$	20	2265	154	359 [3]	491	93	− 71
	C.K. 24	71.8	0.0	None	20	2845	179	0	183	146	−150
				Soy milk	20	2817	175	560	663	145	− 73
	P.T. 27	61.7	−0.45	None	20	2796	182	0	160	100	− 78
				Soy milk	20	2796	174	453	480	135	+ 12
				Soybeans and $CaSO_4 \cdot 2H_2O$	20	2801	160	409 [3]	484	110	− 25
	S.Z. 25	63.6	0.0	None	20	2664	176	0	178	131	−133
				Soy milk	20	2665	168	476	561	126	− 43
				Soybeans and $CaSO_4 \cdot 2H_2O$	20	2671	153	423 [3]	562	117	−103

[1] The last three 5-day periods (total 15 days) were used in computing the daily average calcium metabolism and utili

[2] Inadvertant mixing of samples made use of all 5-day periods (total 20 days) imperative.

[3] The calcium supplement was supplied half in the form of $CaSO_4$ and half as whole soybeans.

[4] Subjects P.T. and S.Z. served on all three diet squads. Complete data should be obtained from only five of the or members of Diet Squads II and III. S.Z. replaced one of the original members of Diet Squad II and did not participate $CaSO_4$ study with this group. One member of Diet Squad III dropped from the squad after the basal diet and hence di complete either the Mull-soy or whole-soybean experiment. Only four of the subjects completed the whole soybean experiment

These experiments again show the great variability exhibited by individual subjects (see Steggerda and Mitchell, '46) and suggest that average per cent utilizations of calcium should not be regarded as absolute values but rather serve to show differences in the availability of calcium in the products studied.

SUMMARY

The utilization of the calcium in three foods and one pure chemical compound was studied in adult human subjects. The supplementary calcium consumed ranged from 319 to 795 mg per day. The average per cent utilization of the calcium in evaporated milk was found to be 29.1; in calcium sulfate, 23.7; in soybean "milk," 22.6; whole cooked soybeans, 10.4.

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VITAMIN Bc AND ITS RELATION TO β-PYRACIN LACTONE IN THE METABOLISM OF THE CHICK

C. J. CAMPBELL, R. A. BROWN, O. D. BIRD AND A. D. EMMETT
The Research Laboratories, Parke, Davis and Company, Detroit, Michigan

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INTRODUCTION

Hogan and Parrott ('40) postulated the existence of vitamin Bc after observing the curative effect of liver extracts in correcting both anemia and poor growth in chicks raised on semi-purified rations. Later, Pfiffner and associates ('43), who isolated this vitamin in crystalline form, presented evidence that it would replace liver extracts when incorporated in the basal diet. These workers retained the term vitamin Bc for this compound. In supplementing these findings Campbell et al. ('44a, b, '45) were of the opinion that relatively large amounts of this vitamin were specifically needed to prevent macrocytic, hypochromic or hyperchromic anemia, leukopenia, thrombocytopenia, poor growth, and poor feathering in chicks. On the other hand, Briggs, Luckey, Elvehjem and Hart ('43, '44, '45), using crude concentrates of liver, concluded that "normal chicks may be obtained with 8 gamma of vitamin Bc activity, or less, per 100 gm of ration." In addition, Hill et al. ('44) stated that "the folic acid (vitamin Bc) requirement of the chick for growth and the prevention of anemia appears to be less than 15 gamma per 100 gm of diet."

Another and similar factor, active in the nutrition of the chick, namely the L. casei factor from a fermentation residue, was isolated in pure form by Hutchings and co-workers ('44).

Although its absorption spectrum appears to be very similar to that of vitamin Bc (Bloom et al., '44), Scott, Norris, Heuser, and Bruce ('45) showed that α or β pyracin had to be included with it in a purified chick ration to prevent completely macrocytic, hypochromic anemia. They also found that the β-pyracin lactone was more effective than the α-pyracin lactone in promoting the growth of chicks. The observations were confirmed by Daniel, Scott, Norris and Heuser ('45). These workers concluded that folic acid (vitamin Bc) may be an addition product of pyracin and the L. casei factor; or that an enzyme system, requiring pyracin and the L. casei factor, is related to the formation of folic acid.

Unfortunately we have not had sufficient quantities of the above mentioned factors in crystalline form to conduct parallel experiments. We desire, however, to present some data which indicate first the essential role of vitamin Bc in the nutrition of the chick and secondly, the ineffectiveness of the β-pyracin lactone in supplementing vitamin Bc.

EXPERIMENTAL

Day-old White Leghorn chicks, hatched from eggs obtained from year-old hens which had been kept in close confinement and raised on dry feeds, were weighed, banded, and segregated into groups of ten each. They were housed in metal brooders having raised screen bottoms. The temperature of the air-conditioned room was maintained at 90°F. for the first week of life and then reduced by 5°F. each succeeding week on test until the temperature reached 75°F. The food and water were given ad libitum. The composition of the diets is given in table 1. In addition, a control group of chicks was given a commercial broiler feed[1] enriched with 3% desiccated whole pork liver and 3% dried brewers yeast.

The chicks were weighed periodically, and examined critically for perosis and feather development. An arbitrary scoring system was devised to help evaluate these conditions. If no evidence of perosis existed, no score was indicated. A

[1] Larrowe Milling Co.

slight puffiness of the leg joint without actual slipping of the tendon was scored 1. The most advanced stage of perosis was marked 4. In the accompanying tables the average perotic values represent the score per leg and not per bird. Likewise, feathering was graded in six steps, the normal condition was designated as 0, the poorest feathering as 6. As the development was bilaterally symmetrical, the average represents each bird rather than each wing.

Vitamin Bc crystals (isolated from liver) were dissolved in aqueous 0.005 N NaOH and stored in the refrigerator at

TABLE 1

Composition of basal diets.

INGREDIENTS	NO. 59744	NO. 96355	NO. 96365	NO. 96375
Cornstarch — gm	52.3	52.3	51.3	51.3
Purified casein — gm	25.0	25.0	25.0	25.0
Gelatin — gm	10.0	10.0	10.0	10.0
Cellu flour — gm	3.0	3.0	3.0	3.0
Salt mixture, Jones and Foster ('42) — gm	5.0	5.0	5.0	5.0
$MnSO_4 : 4H_2O$ — gm	0.1	0.1	0.1	0.1
Succinylsulfathiazole — gm	..	..	1.0	1.0
ADEK mixture [1] — gm Vit. A 7520 USP units Vit. D (natural) 725 USP units mixed tocopherol 32 mg menadione — 100 μg	0.1	0.1	0.1	0.1
l-cystine — gm	0.3	0.3	0.3	0.3
Lard — gm	3.9	3.9	3.9	3.9
Choline chloride — gm	0.2	0.2	0.2	0.2
Thiamine [2] — mg	0.4	0.4	0.4	0.4
Riboflavin [2] — mg	0.8	0.8	0.8	0.8
Pyridoxine [2] — mg	0.6	0.6	0.6	0.6
Nicotinic acid [2] — mg	2.0	2.0	2.0	2.0
Inositol [2] — mg	0.05	0.05	0.05	0.05
Sodium pantothenate — mg	1.1	1.1	1.1	1.1
Biotin — mg	0.02	0.02	0.02	0.02
PABA [2] — mg	15.0	..	15.0	..

[1] Added to the diet for the first week of test. Thereafter, these fat soluble vitamins were fed orally as follows. Per 0.1 ml corn oil., per chick per week: vitamin A, 1600 USP units; vitamin D (natural) 160 USP units; α-tocopherol, 6 mg; and menadione, 0.5 mg.

[2] These B vitamins are mixed in a small amount of lactose (total weight 0.1 gm per 100 gm ration).

0°C. When the chicks required fresh ration about once a week, new lots were made from this stock solution. The lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxy-methylpyridine, termed β-pyracin lactone by Daniel et al. ('45) also was dissolved in water before mixing in the rations. In one experiment, an aqueous solution of the lactone was given daily by pipette to each chick. This solution was made up once a week and stored in the refrigerator. The yeast extract, no. 38843, sometimes referred to as the reference standard, was an aqueous extract of plasmolyzed brewers yeast which had been dried and powdered. Two previous biological chick assays of this material, using crystalline vitamin Bc as a standard, gave values of 54 and 50 gamma of vitamin Bc per gm, respectively.

Experiment no. 1 (vitamin Bc)

The object of this test was to determine whether or not crystalline vitamin Bc would complete the nutritional requirements of the growing chick when fed in combination with the previously known necessary dietary factors. The results are summarized in table 2. After 38 days of feeding the birds receiving 25 gamma of crystalline vitamin Bc per 100 gm of ration weighed 393 gm and a similar group receiving an equivalent amount of vitamin Bc as a yeast extract weighed 355 gm. This is considered to be a normal growth range for these birds.

Experiment no. 2 (vitamin Bc with β-pyracin lactone)

The second test was designed to determine whether or not β-pyracin lactone would supplement vitamin Bc in the nutrition of the chick. Three groups of chicks receiving 15, 50, or 150 μg of β-pyracin lactone per 100 gm of diet, respectively, were run as controls. A fourth group received 50 μg of β-pyracin lactone together with 15 μg of vitamin Bc per 100 gm of ration. This level of vitamin Bc was known to be suboptimum in its action on growth and hemopoiesis. The results are reported in table 3.

TABLE 2

Influence of vitamin Bc on chick growth.

LEVEL OF VIT. BC PER 100 GM RATION [1]	NO. OF CHICKS ♂	NO. OF CHICKS ♀	AVERAGE WEIGHT (GM) DAYS ON RATION 0	13	20	31	38
I. Vitamin B_c crystals no. 91975							
0 μg	1	4	41	90	107	140	153
5 μg	3	3	40	93	126	151	174
15 μg	3	6	39	94	135	222	243
25 μg	6	0	41	107	180	306	393
II. Yeast extract no. 38843							
5 μg [2]	4	4	42	99	133	183	219
15 μg [2]	5	5	39	94	130	189	252
25 μg [2]	9	1	39	104	154	262	355
200 μg [2]	10	0	38	117	176	322	419

[1] Basal ration no. 59744.

[2] These are equivalent vitamin Bc levels according to previous chick bioassay results. See text for description of no. 38843.

TABLE 3

The ineffectiveness of β-pyracin lactone on the vitamin Bc syndrome in chicks.

NATURE OF TEST GROUPS	TREATMENT LEVEL PER 100 GM OF RATION (μg)	NO. CHICKS Started	NO. CHICKS Survived	AVERAGE BODY WEIGHT 3 wk.	AVERAGE BODY WEIGHT 27 da.	4-WEEK DATA Perosis score per leg	Feathering score	Hematocrit (vol. %)	Hemoglobin (gm %)
Basal diet, no. 59744	..	10	8	116	134	3.2	5.3	18.4	5.33
β-Pyracin Lactone	15	10	6	97	110	3.3	6.0	16.5	4.54
β-Pyracin Lactone	50	10	8	104	119	3.2	5.3	18.8	5.87
β-Pyracin Lactone	150	10	8	89	95	3.6	6.0	15.3	4.09
Vitamin Bc crystals	15	10	9	133	171	2.9	4.7	28.1	8.02
Vitamin Bc crystals + β-Pyracin Lactone	15 + 50	10	9	125	173	3.1	4.8	25.0	7.36
Reference Standard (Aqueous extract of yeast). no. 38843	5 [1]	10	9	118	144	3.6	5.9	22.7	6.40
	15 [1]	10	8	135	157	3.3	4.9	26.5	7.77
	30 [1]	10	8	186	219	3.3	3.3	31.6	8.67

[1] Based on chick assay. See footnote table 2.

Three β-pyracin lactone control groups fed the basal diet plus the levels of 15, 50 or 150 μg per 100 gm of ration showed no increase in growth response or improvement in hemopoiesis when compared with the chicks receiving the basal diet.

Vitamin Bc at 15 μg per 100 gm of ration produced chicks which averaged 171 gm per group after 27 days on test. The addition of the lactone produced no measurable increase in growth nor change in perosis, feathering or blood picture. Therefore, it was concluded that no biological activity could be attributed to β-pyracin lactone under the conditions noted in this experiment.

Experiment no. 3 (vitamin Bc, β-pyracin lactone and succinylsulfathiazole)

Since the foregoing tests indicated that β-pyracin lactone had no supplemental effect on our regular anemia-producing ration, the possibility still existed that under similar conditions succinylsulfathiazole, with or without p-aminobenzoic acid, might have some influence on the vitamin Bc syndrome in chicks. Accordingly, ration no. 59744 was modified to contain 1% of the sulfa compound (table 1). The lactone was given by pipette to each chick at a level of 5 μg per day which was equivalent to 50 to 60 μg per 100 gm of ration based on the average food intake for these animals of 8 to 10 gm per day. Further, in order to demonstrate more clearly the lack of supplemental action of β-pyracin lactone, a lower level of vitamin Bc, namely 10 μg per 100 gm of ration, was employed. By these modifications a dietary regime was produced which was similar to the one used by Scott et al. ('45).

In table 4 the resultant data are given. In confirmation of experiment no. 2, above, β-pyracin lactone showed no antianemia or growth activity when fed in conjunction with crystalline vitamin Bc in our regular ration, no. 59744, or in the succinylsulfathiazole basal ration (with or without PABA).

TABLE 4

Growth and hemoglobin-promoting response to crystalline vitamin Bc in chick diets with and without sulfa drugs.

DESCRIPTON OF TEST GROUP	VITAMIN BC LEVEL/100 GM OF RATION (μg)	NO. CHICKS		3-WEEK DATA		
		Started	Surviving [1]	Body weight (gm)	Hematocrit (vol. %)	Hemoglobin (gm %)
A. Ration no. 59744 — with PABA, without succinylsulfathiazole						
(a) Basal ration	0	11	7	97	15.3	4.50
(b) β-pyracin lactone [2]	0	10	5	79	15.2	4.41
(c) β-pyracin lactone [2] + Vit. Bc cryst.	10	10	8	110	16.1	4.83
(d) Vit. Bc cryst.	10	10	7	110	19.0	5.25
(e) Vit. Bc cryst.	25	10	10	154	28.7	7.74
(f) Yeast extract [3]	10	10	9	136	19.9	5.45
(g) Yeast extract [3]	25	10	10	140	25.9	7.37
(h) Yeast extract [3]	40	10	10	182	30.0	7.68
B. Ration no. 96355 — without PABA and without succinylsulfathiazole						
(a) Basal ration	0	10	4	80	16.5	4.22
(b) Vit. Bc cryst.	10	10	7	111	16.0	4.51
(c) Vit. Bc cryst.	25	10	10	153	27.9	7.50
(d) Yeast extract [3]	10	10	8	104	18.9	5.56
(e) Yeast extract [3]	25	10	8	136	25.8	7.13
C. Ration no. 96965 — with PABA and succinylsulfathiazole						
(a) Basal ration	0	10	6	94	19.0	5.45
(b) Vit. Bc cryst.	10	10	9	118	20.7	5.90
(c) Vit. Bc cryst.	25	10	8	171	29.5	8.18
(d) Yeast extract [3]	10	10	9	122	18.7	4.80
(e) Yeast extract [3]	25	10	10	146	23.7	6.63
D. Ration no. 96375 — without PABA, with succinylsulfathiazole						
(a) Basal ration	0	10	4	70	13.0	3.54
(b) β-pyracin [2]	0	10	5	77	13.8	3.78
(c) β-pyracin [2] + vit. Bc cryst.	10	10	9	101	20.2	5.28
(d) Vit. Bc cryst.	10	10	10	114	18.8	4.92
(e) Vit. Bc cryst.	25	10	10	144	26.2	7.18
(f) Yeast extract [3]	10	10	9	94	19.1	5.16
(g) Yeast extract [3]	25	10	10	131	24.4	6.37
E. Commercial broiler Ration, fortified [4]	105	10	9	155	29.4	7.92

[1] Age of chicks — 3 weeks.

[2] 5 μg per chick per day.

[3] No. 38843. See footnote table 2.

[4] 3% whole desiccated pork liver (Wilson's) plus 3% dried brewers yeast (Anheuser-Busch, strain G) added.

DISCUSSION

The foregoing data confirm earlier conclusions from this laboratory, presented by Pfiffner and associates ('43) and Campbell et al. ('44a, b, '45), that vitamin Bc per se is an important essential in the nutrition of the chick. These findings are in opposition to those of Briggs et al. ('43, '44, '45) and those of Hill, Norris and Heuser ('44). The discrepancy, however, may be explained by the fact that the microbiological assay used by these laboratories did not determine the total amount of vitamin Bc activity but, for the most part, only the free form of the vitamin present in the supplements fed the chicks. For example, in this laboratory microbiological investigation of the vitamin Bc conjugate indicates that treatment of a yeast extract by autoclaving at pH 4 for 12 hours will release only 10 to 15% of the total vitamin present. (Unpublished data — O.D.B.) Likewise, Bird et al. ('45) have shown that vitamin Bc cannot be completely liberated from its conjugate by the taka-diastase and papain digestion procedure of Cheldelin et al. ('42). The possible conclusion, then, is that Briggs and associates ('45) in reality fed their chicks more vitamin Bc than was indicated. On this basis one may conjecture that the B_{11} factor of Briggs et al. ('43) is a vitamin Bc conjugate. Likewise, a similar condition existed with the experiments of Hill et al. ('44). In this work the concentrate fed the chicks contained factors R or S. In each case the factors were found to possess anti-anemic properties. It is probable that there is some vitamin Bc conjugate in these fractions. Moreover, since their data showed that factor S at a level equivalent to 15% yeast was nearly equal in hematopoietic activity to factor R at a level equivalent to 5% yeast, one may estimate that factor R contains at least three times as much vitamin Bc activity as does factor S.

Furthermore, the absolute amount of vitamin Bc activity required by the chick is open to question. For example, our first experiments suggested that for normal response relatively high levels of the vitamin, 100 to 400 μg per 100 gm of ration, were necessary, while our second test (Pfiffner

and others, '45) showed that maximum growth could be obtained with but 25 μg per 100 gm of ration using the free form, or an equivalent amount of vitamin Bc conjugate. Peak blood values were observed at the 200 μg level but this experiment did not prove that a smaller quantity, somewhat greater than 25 μg, would not have been in the optimum range. The work reported in table 2 suggests that 25 μg more nearly approached the optimum requirements for growth. This value is in line with the results of Briggs et al. ('45), who reported that 50 μg of the L. casei factor (type not designated) per 100 gm of ration produced normal growth and normal hemoglobin formation.

In the present tests there are two factors which may have had a bearing on the results: First, on investigating the source of the chicks used, it was noted that the chicks in the first experiment were hatched from pullet eggs, while in the present investigation the chicks came from eggs produced by more mature hens of the same strain of White Leghorns. Secondly, it is possible that seasonal variation may have been a contributing factor. The first experiments were completed in the late fall and early winter while those reported at this time were carried out in the late spring. If a true growth requirement is to be established, the above points should be considered. Actually in our routine vitamin Bc assays of liver and yeast preparations (unpublished data) it has been found that the hematopoietic response is a more reliable index of biological activity than is growth. The variation from season to season and between groups of chicks is not as marked. This fact may indicate that the primary role of vitamin Bc is concerned with the blood forming organs and that growth is of secondary consideration.

Again, the basal ration with and without succinylsulfathiazole and/or p-aminobenzoic acid caused little or no change in the growth of the negative control groups. This is in direct contrast to the results reported by Briggs et al. ('43). These authors reported that growth and feathering on their basal diet was retarded at least 50% by the feeding of suc-

cinylsulfathiazole. The explanation for this difference may be due to our use of corn starch as a source of carbohydrate rather than dextrin. Also, there should be noted in our experiments the possible effectiveness of p-aminobenzoic acid in promoting growth or hemoglobin formation by its stimulation of the intestinal bacteria to produce other unknown factors (table 4).

Finally, in considering the role of β-pyracin lactone in the nutrition of the chick, it is interesting to compare the points of similarity between the published data of Scott et al. ('45) and some of our own data (3-week, table 3). The growth chicks receiving the basal diet plus β-pyracin lactone ranged from 91 to 100 gm as reported by the Cornell investigators while the growth of our chicks ranged from 89 to 104 gm. Both laboratories used chicks which reached approximately the same maximum weight; the Cornell investigators, using a well balanced grain chick ration, obtained birds averaging 192 gm, while our birds receiving the fortified liver-yeast diet, averaged 186 gm at the end of 3 weeks.

If the analogy between the two investigations is carried farther, it may be noted that in our laboratory where 50 μg of β-pyracin lactone and 15 μg of vitamin Bc were used per 100 gm of ration, a growth of 125 gm was obtained by the end of 3 weeks, whereas the Cornell authors, using 25 μg of the lactone and 25 μg of the L. casei factor (LCF) reported a body weight of 105 gm. When they doubled the quantity of LCF and β-pyracin lactone (50 μg of each per 100 gm of ration), they obtained a growth of 144 gm in the 3-week period. This would indicate that 15 μg of vitamin Bc gives a growth response intermediate between 25 μg and 50 μg of the LCF. Since we have already indicated in experiment 2 that β-pyracin lactone had no supplementary effect on vitamin Bc and the Cornell investigators have reported that β-pyracin lactone supplements the LCF, this suggests that the LCF is not identical with vitamin Bc compound isolated from liver. The validity of this reasoning can only be proven when the LCF (fermen-

tation), β-pyracin lactone and vitamin Bc compounds are compared simultaneously in the same laboratory.

In this connection it is interesting to note that Daft and coworkers ('45) have also discussed the possibility of the difference between a fermentation L. casei factor and the liver L. casei factor; and that Day and others ('44a, b) have proven the conjugate nature of the fermentation product.

CONCLUSIONS

Based upon the experimental conditions as carried out in these laboratories the following conclusions are warranted.

1. Crystalline vitamin Bc is required by the chick for normal growth, feathering and hemopoiesis. This confirms our previous findings.

2. In the presence of a sub-optimal amount of crystalline vitamin Bc, β-pyracin lactone has no supplemental effect on growth, feathering and hemopoiesis of the chick, even in the presence of succinylsulfathiazole (with or without PABA). This suggests that L. casei factor from a fermentation residue is not the same as vitamin Bc.

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THE NUTRITIVE VALUE OF CANNED FOODS

CHANGES IN THE VITAMIN CONTENT OF FOODS DURING CANNING [1]

N. B. GUERRANT, M. G. VAVICH,[2] O. B. FARDIG, R. A. DUTCHER AND R. M. STERN

The Pennsylvania State College, State College, Pennsylvania; National Canners Association, Washington, D. C.

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The vitamin content of canned foods has been a matter of increasing interest during the past 20 years. The results of earlier investigations showed that many canned foods are relatively good sources of some of the vitamins. The more recent studies of Pressley, Ridder, Smith and Caldwell ('44), Ives, Wagner, Elvehjem and Strong ('44), Thompson, Cunningham and Snell ('44), and Ives, Zepplin, Ames, Strong and Elvehjem ('45) have further indicated the contribution that canned foods make in supplying the human vitamin requirements. This extensive series of studies covered more than thirty different food products and involved over 800 samplings of canned foods of known origin (Clifcorn, '44). In addition to indicating the relative richness of the various canned foods with respect to the several vitamins, the data reported also indicate a rather wide variation in the amounts of these vitamins found in different samplings of the same

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[2] Present address School of Agriculture, University of Arizona, Tucson, Arizona.

product. Whether such variations were due to differences in the variety of the particular product canned, to differences in soil or climatic conditions under which the products were grown, to differences in the procedure employed in canning, or to a combination of these factors could not be ascertained from the data reported by the above authors. It seems reasonable, however, to suspect that some of the variations in vitamin content were at least partially due to differences in canning methods. It also seems logical to suspect that, if those canning conditions could be ascertained which are conducive to extensive vitamin losses, modification of the methods might be effected which would result in the production of more nutritious canned products.

A number of investigators have reported data which indicate that the vitamin content of canned foods is adversely affected by various conditions encountered previous to and during processing. Among these are: Fellers, Esselen and Fitzgerald ('40); Adams ('42, '44), Ross ('44); Clifcorn and Heberlein ('44); Greenwood, Kraybill, Feaster and Jackson ('44); Moore, Wiederhold, Atkins and McDowell ('44); Wagner, Ives, Strong and Elvehjem ('45); Robinson, Stotz and Kertesz ('45); and Poe and McGuire ('45).

From the contents of the above reports there is ample justification for continuing researches along these lines in an effort to determine some of the factors or conditions associated with food canning procedures which are conducive to high vitamin retention, in order that canning methods may be improved to the point of furnishing more nutritious canned foods. The present studies were conducted with the hope of obtaining additional information regarding these questions. Collaboration and cooperation of various canners in the states of New York, Delaware and Maryland during the canning seasons of 1943 and 1944 made possible a study of the effects of some of the various steps in commercial canning procedures on the vitamin losses from nine vegetables and one fruit. While the investigation involved cooperation with canneries located in the three states, the major portion of the investiga-

tion relating to canning conditions was carried out in western New York.[3]

EXPERIMENTAL

The actual technical studies at the canneries involved the procuring of representative samples at various points along the canning line, the determination of ascorbic acid and the preparation of appropriate samples for the assay of carotene, thiamine, riboflavin, niacin or for total solids. These latter determinations were carried out in the home laboratory at a later date. The various technical operations at the several plants were greatly facilitated by means of mobile laboratory equipment which could be readily transported from plant to plant. In every instance the canning procedure was the one usually in practice in the plant under consideration. An attempt was made to study canning operations in as many different plants as possible, regardless of plant capacity or peculiarity of canning practices. Canning operations in twenty-seven different plants were investigated. Some plants were examined with respect to a single survey involving one product while other plants were investigated with respect to as many as five different products, involving one or more surveys with each product. Two types of surveys were conducted: (1) an "over-all" survey where the product was sampled as it entered the canning line and again as it emerged as a finished product and (2) a "stage" survey where samples were taken at various points along the canning line, including the finished product.

The products studied were: asparagus (spears and center cuts), green beans (cut and whole), wax beans (cut), lima beans, carrots (diced), whole kernel yellow corn (brine- and vacuum-packed), peas (sweet and Alaska), tomatoes, tomato juice and red sour cherries (pitted). In each instance an

[3] The authors are especially indebted to the officers and the staff of the Research Laboratory of Birdseye-Snider Division, General Foods Corporation, Albion, N. Y., who kindly placed laboratory facilities at the disposal of the authors and who also made many of the contacts and arrangements for cooperative studies at the various canneries in that area.

effort was made to secure data concerning the previous history of the product, with special reference to variety, date of planting, soil type, type of fertilization, growing conditions, harvesting date and yield per acre. The method of blanching, blanching temperature and blanching time were also recorded. In many instances it was possible to follow the product from the time it left the farm until it had passed through the entire canning procedure.

Methods of sampling

A uniform batch of the raw product of sufficient size to yield at least 300 pounds of the canned food constituted each sampling. Preliminary studies were carried out in each plant for the purpose of determining the time required for each product to pass from one sampling point to the succeeding point. Without altering the usual canning procedure, the experimental batch was introduced into the canning line at some time during the day when there was a break in the continuity of operation such as that which usually occurs when it is necessary to change from one variety or grade of product to another or immediately following the lunch hour.

The samples which were taken at the various stations along the canning line consisted of at least 2 kg of the material. To secure the final sample twelve no. 2 or no. 2½ cans or six no. 10 cans of the canned product were used. Half of the cans were opened and sampled immediately after cooling, while the remaining cans were stored at a low temperature until subsequently examined at the home laboratory. Samples for ascorbic acid determinations were stabilized with metaphosphoric acid and assayed immediately, while samples for carotene, thiamine, riboflavin and niacin determinations were stabilized by methods which will be described later. All stabilized samples were stored under refrigeration until the vitamin analyses had been completed.

Representative samples for total solids determinations were prepared by placing equal weights of the product and of distilled water in a no. 2 can, closing the can and sterilizing

by autoclaving. Total solids were subsequently determined in the home laboratory by pureeing the contents of the can in a Waring Blendor and reducing a weighed aliquot to constant weight in a Brabender Moisture Tester which was operated at 85°F.

Methods of vitamin assay

Ascorbic acid was determined immediately after sampling by direct titration with 2, 6-dichlorobenzenone-indophenol according to a procedure previously described by Vavich, Stern and Guerrant ('45) for peas, except for the following modifications which were found necessary in the case of fresh asparagus and sour cherries, owing to the presence of interfering pigments. The metaphosphoric extract of fresh unblanched asparagus was found to contain a pinkish coloration which interfered with the usual direct titration procedure. This pigmentation appears to be destroyed by heat so that the determination of the ascorbic acid content of blanched or processed asparagus by direct dye titration is not a serious problem. To determine the ascorbic acid content of fresh asparagus, different but measured amounts of the dye solution were added to each of a series of aliquots of the asparagus extract. Sufficient dilute NaOH solution was then added to each aliquot to bring the pH to about 6.5. At or above this pH an excess of the indophenol dye changes to form a blue coloration. Using the presence or absence of the blue coloration as the criterion, it was possible to determine the proper end-point.

The extraction of a red coloration from sour cherries by the metaphosphoric acid solution also made it impossible to use the direct titration method for determining the ascorbic acid content of this product. This red pigment, however, is not extracted from metaphosphoric acid solution by xylene. It was possible, therefore, to determine the proper titration value by measuring the smallest volume of the indophenol dye solution that would produce a pinkish coloration in the xylene layer when the cherry extract-dye solution was shaken with

xylene. Obviously this pinkish coloration is due to an excess of the dye and, therefore, indicates the end-point.

Samples for thiamine, riboflavin and niacin assays were stabilized in N/10 H_2SO_4, with chloroform and toluene as added preservatives, while samples for carotene assays were stabilized by blending into a puree in the presence of alkaline ethyl alcohol in accordance with the recommendations reported by Feaster and Alexander ('44). The methods employed in assaying carotene, thiamine, riboflavin and niacin were essentially the same as those described in an earlier publication by Guerrant, Vavich and Fardig ('45). Since all of the products studied were not considered rich sources of all of the vitamins, they were assayed only for those vitamins which they were known to contribute in substantial amounts to the human diet. While the samples for carotene, thiamine, riboflavin and niacin assays were stored under refrigeration pending analysis, every effort was made to complete the vitamin assays at the earliest possible date. With the majority of the samples this was accomplished within 10 days or 2 weeks from the dates the samples were taken.

Data

The data obtained in this series of studies have been condensed so far as general characteristics will permit and are presented in table 1.

DISCUSSION

It was obvious that all of the detailed data could not be included, hence condensations and deletions were essential. Among some of the various factors involved in the study such as variety of product, soil type, growing season, yield per acre, canning plant, manufacturer's grade, can size and station of sampling, the data indicated that variety, can size and station of sampling were the most important insofar as their effects on vitamin retention were concerned. For this reason, these latter factors have been retained in the tabulation (table 1) and the data have been condensed without regard

to soil type, fertilization, growing season, yield per acre, canning plant, or manufacturer's grade. It is hoped that these supplementary data may be published in an appropriate form at a later date.

Since there were frequently moisture additions or losses during the various stages of canning, vitamin values calculated to the "as assayed" basis are not necessarily true measures of vitamin retention or vitamin losses. In consequence, vitamin potencies were also calculated on the basis of "dry weight." However, in this calculation, no correction was made to compensate for the added sugar and salt. Since appreciable amounts of water-soluble substances are frequently lost during canning, especially during blanching in water, vitamin values expressed in terms of dry weight are also lacking in finality so far as they reveal the actual effect of the canning operation on the vitamin content of the finished product. Inasmuch as the vitamin content of canned foods is usually expressed in terms of "as assayed" or on the "dry weight" basis, both values are included in the above tabulation. Supplementary studies are now under way in an attempt to determine whether other constituents of canned foods (alcohol insoluble solids, crude fiber and calcium) constitute a more reliable basis for computing vitamin retention.

Asparagus

Two samplings of asparagus were investigated. The samplings consisted of spears and center cuts, packed by the same canner on the same day and from the same batch of raw stock. Each sampling of asparagus was steam blanched for 1.25 minutes, spray rinsed, sorted and hand packed in cans, the spears being packed in no. 2 cans and the center cuts in no. 10 cans. After adding salt, the cans were steam exhausted, sealed, processed in a vertical retort at 248°F. for 15 minutes for the no. 2 cans and for 35 minutes for the no. 10 cans and canal cooled.

It may be noted from the data presented in table 1 that the center-cut asparagus originally contained about one-half

TABLE 1

Changes in the vitamin content of canned foods during preparation and processing.

PRODUCT	VARIETY	CAN SIZE	STATION OF SAMPLING	NUMBER OF SAMPLES	VITAMIN CONTENT (MG/100 GM)									
					Ascorbic acid		Carotene		Thiamine		Riboflavin		Niacin	
					As assayed	Dry wt.[1]	As assayed	Dry wt.[1]	As assayed	Dry wt.[1]	As assayed	Dry wt.[1]	As assayed	Dry wt.[1]
Asparagus (Spears)	Unknown	2	Before blancher	1	50.6	538	0.62	6.5	0.23	2.5	0.16	1.7	1.74	19.3
			After canning	1	22.3	297	0.51	6.8	0.12	1.6	0.14	1.8	1.50	20.1
Asparagus (Center cuts)	Unknown	10	Before blancher	1	26.0	325	0.26	3.2	0.14	1.8	0.08	1.0	1.02	13.4
			After canning	1	11.5	164	0.19	2.7	0.08	1.0	0.06	0.8	0.88	13.2
Beans (Whole green)	Refugee	2	After snippers	7	10.1	101	0.21	2.4	0.11	1.3	...	..	...	...
			After canning	7	2.5	44	0.13	2.2	0.04	0.7	...	..	...	...
Beans (Whole green)	Tendergreen	2	After snippers	1	6.8	81	0.46	5.5	0.07	0.9	...	..	...	...
			After canning	1	1.7	35	0.26	5.3	0.03	0.5	...	..	...	...
Beans (Cut green)	Refugee	2	After snippers	3	11.9	115	0.23	2.3	0.10	1.0	...	..	...	...
			After canning	3	3.8	62	0.11	2.0	0.04	0.6	...	..	...	..
Beans (Cut green)	Refugee	10	After snippers	3	14.6	141	0.20	2.1	0.11	1.1	...	..	...	...
			After canning	3	4.5	68	0.12	1.7	0.05	0.7	...	..	...	...
Beans (Cut green)	Bountiful	2	After snippers	1	18.6	221	0.39	4.6	0.11	1.3	...	..	...	...
			After canning	1	4.8	91	0.20	3.9	0.03	0.6	...	..	...	...
Beans (Cut green)	Tendergreen	2	After snippers	1	6.3	75	0.29	3.5	0.11	1.4	...	..	...	...
			After canning	1	2.3	45	0.16	3.0	0.04	0.7	...	..	...	...
Beans (Cut green)	Tendergreen	10	After snippers	2	13.3	134	0.32	3.2	0.10	1.1	...	..	...	...
			After canning	2	3.9	67	0.16	2.7	0.04	0.7	...	..	...	...
Beans (Cut green)	Refugee	2	After snippers	1	11.2	113	0.21	2.1	0.10	1.0	...	..	...	...
			After cutters	1	10.9	127	...	..	0.10	1.2	...	..	...	...
			After blancher	1	7.3	92	0.14	1.8	0.09	1.1	...	..	...	...
			After filler	1	7.3	91	...	..	0.08	1.0	...	..	...	...
			After canning	1	3.4	59	0.10	1.7	0.04	0.7	...	..	...	...
Beans (Cut green)	Refugee	10	After snippers	1	13.2	147	0.20	2.2	0.10	1.2	...	..	...	...
			After cutters	1	13.5	150	...	..	0.11	1.2	...	..	...	...
			After blancher	1	12.1	138	0.20	2.3	0.11	1.3	...	..	...	...
Beans	Round pod kidney	2	After snippers	2	9.0	103	0.07	0.8	0.11	1.3	...	..	...	...
			After cutters	2	8.7	98	...	..	0.10	1.2	...	..	...	...

			After canning	1	3.4	59	0.10	1.7	0.04	0.7	...	..	...	...
Beans (Cut green)	Refugee	10	After snippers	1	13.2	147	0.20	2.2	0.10	1.2	...	..	...	...
			After cutters	1	13.5	150	...	..	0.11	..	...	..	...	...
			After blancher	1	12.1	122	0.09	0.9	0.11	1.1	...	..	...	...
Beans (Cut wax)	Round pod kidney	2	After snippers	2	9.0	103	0.07	0.8	0.11	1.3	...	..	...	...
			After cutters	2	8.7	98	...	..	0.10	1.2	...	..	...	...
			After blancher	2	7.1	85	0.05	0.6	0.09	1.2	...	..	...	...
			After filler	2	7.1	83	...	..	0.09	1.0	...	..	...	...
			After canning	2	3.4	59	0.03	0.5	0.04	0.7	...	..	...	...
Beans (Lima)	Henderson Bush	2	After viner	1	10.1	21	...	..	0.30	0.6	0.09	0.2	1.33	2.7
			After canning	1	3.0	13	...	..	0.06	0.3	0.04	0.2	0.50	2.2
Beans (Lima)	Henderson Bush	10	After viner	1	10.1	21	...	..	0.30	0.6	0.09	0.2	1.33	2.7
			After canning	1	3.2	13	...	..	0.04	0.2	0.03	0.1	0.52	2.1
Beans (Lima)	Clark's Allgreen	2	After viner	2	28.7	89	...	..	0.23	0.7	0.16	0.5	1.53	4.7
			After blancher	2	22.0	71	...	..	0.15	0.5	0.15	0.5	1.31	4.2
			After filler	2	9.7	63	...	..	0.08	0.5	0.08	0.5	0.70	4.4
			After canning	2	10.1	66	...	..	0.05	0.3	0.08	0.5	0.66	4.2
Carrots	Chantenay	10	After washer	2	3.9	36	19.90	187.5	0.07	0.6	...	..	0.56	5.3
			After blancher	2	3.0	28	18.70	173.4	0.07	0.6	...	..	0.50	4.6
			After filler	2	3.0	28	....	....	0.07	0.5	...	..	0.48	4.4
			After canning	2	3.2	39	12.80	154.6	0.04	0.5	...	..	0.36	4.3
Cherries	Montmorency	10	From rec. tank	6	8.9	50	0.74	3.9	...	..	...	..	...	..
			After canning	6	5.8	48	0.61	4.7	...	..	...	..	...	..
Corn	Golden Cross	2	After cutters	4	9.4	29	0.22	0.6	0.16	0.5	0.08	0.2	1.83	5.6
			After washer	4	7.4	25	...	..	0.15	0.4	0.08	0.2	1.61	5.4
			After filler	4	5.7	22	0.21	0.7	0.10	0.4	0.06	0.2	1.30	4.8
			After canning	4	5.8	26	0.13	0.6	0.03	0.1	0.05	0.2	1.04	5.0
Corn (Brine packed)	Golden Cross	10	After cutters	1	9.4	27	...	..	0.14	0.4	0.08	0.2	1.46	4.3
			After canning	1	7.0	30	...	..	0.02	0.1	0.06	0.2	0.96	4.1
Corn (Brine packed)	Top Cross	2	After cutters	1	9.3	29	...	..	0.16	0.5	0.09	0.3	1.54	4.7
			After canning	1	6.8	28	...	..	0.04	0.2	0.07	0.3	0.84	3.4
Corn (Brine packed)	Tendergold	2	After cutters	1	7.8	24	0.16	0.5	0.13	0.4	0.07	0.2	1.62	5.0
			After canning	1	5.9	27	0.09	0.5	0.03	0.1	0.05	0.2	0.90	4.1
Corn (Vacuum-packed)	Golden Cross	307 x 306	After cutters	2	10.3	31	0.30	0.8	0.16	0.5	0.11	0.3	1.84	5.6
			After washer	2	10.0	30	...	..	0.14	0.5	0.10	0.3	1.71	5.5
			After filler	2	9.4	31	...	..	0.11	0.4	0.10	0.3	2.01	6.4
			After canning	2	10.1	32	0.20	0.7	0.06	0.2	0.09	0.3	1.63	5.1

TABLE 1 *(Continued)*

PRODUCT	VARIETY	CAN SIZE	STATION OF SAMPLING	NUMBER OF SAMPLES	VITAMIN CONTENT (MG/100 GM)									
					Ascorbic acid		Carotene		Thiamine		Riboflavin		Niacin	
					As assayed	Dry wt.[1]	As assayed	Dry wt.[1]	As assayed	Dry wt.[1]	As assayed	Dry wt.[1]	As assayed	Dry wt.[1]
Corn (Vacuum-packed)	Top Cross	307 x 306	After cutters	2	9.1	27	0.20	0.7	0.16	0.5	0.11	0.3	1.84	5.9
			After washer	2	7.2	24	...	..	0.15	0.5	0.11	0.3	1.61	5.3
			After filler	2	5.6	18	0.20	0.7	0.11	0.4	0.10	0.3	1.53	4.7
			After canning	2	6.8	22	0.20	0.7	0.05	0.1	0.10	0.3	1.44	4.7
Peas (Sweet)	Surprise	2	After viner	5	26.2	129	0.48	2.3	0.49	2.4	0.12	0.6	2.81	13.6
			After canning	5	9.8	67	0.34	2.3	0.18	1.2	0.11	0.6	1.53	10.4
Peas (Sweet)	Surprise	2	After viner	2	28.6	144	0.50	2.5	0.52	2.6	0.11	0.6	2.63	13.2
			Before blancher	2	28.1	139	...	..	0.51	2.5	0.11	0.5	2.61	12.9
			After rinse	2	15.1	83	0.48	2.6	0.36	1.9	0.11	0.6	2.50	13.5
			After filler	2	15.7	80	...	..	0.35	1.8	0.10	0.5	2.31	11.8
			After canning	2	9.4	67	0.34	2.4	0.15	1.0	0.10	0.7	1.95	13.8
Peas (Sweet)	Perfection	2	After viner	2	28.4	125	0.59	2.6	0.61	2.7	0.12	0.6	3.36	14.8
			After canning	2	10.3	76	0.47	3.2	0.21	1.5	0.09	0.7	1.27	9.2
Peas (Sweet)	Laxton	2	After viner	1	22.3	96	0.48	2.1	0.59	2.5	0.11	0.5	3.45	14.8
			After canning	1	11.3	70	0.37	2.3	0.22	1.4	0.10	0.6	1.24	7.7
Peas (Alaska)	Super-Alaska	2	After viner	1	33.3	162	0.46	2.4	0.30	1.5	0.13	0.6	2.72	13.2
			After canning	1	10.7	71	0.31	2.1	0.10	0.7	0.06	0.4	0.86	5.7
Peas (Alaska)	Super-Alaska	10	After viner	1	33.3	162	0.49	2.4	0.30	1.5	0.13	0.6	2.72	13.2
			After canning	1	10.7	74	0.30	2.1	0.10	0.7	0.06	0.4	0.87	6.0
Spinach	Heavy Pack	2	After washer	1	40.5	524	3.94	51.2	0.08	1.1	0.20	2.6	0.42	5.5
			After canning	1	22.5	304	3.90	53.2	0.02	0.3	0.10	1.4	0.40	2.7
Spinach	Heavy Pack	10	After washer	4	40.1	562	3.50	52.0	0.09	1.3	0.22	3.1	0.56	8.0
			After blancher	4	39.0	436	...	...	0.09	1.0	0.22	2.5	0.60	7.1
			After filler	4	27.3	357	3.56	52.4	0.08	1.0	0.20	2.9	0.47	6.6
			After exhaust	4	20.4	303	...	...	0.06	0.9	0.18	2.3	0.44	6.1
			After canning	4	24.5	348	3.46	53.6	0.02	0.3	0.18	2.5	0.43	6.2
Tomatoes	Stokesdale	2	After peeler	2	16.2	275	0.65	10.8	0.07	1.1	0.04	0.7	0.55	9.3
			After canning	2	16.9	294	0.55	9.6	0.06	1.1	0.04	0.7	0.54	9.4
			[illegible]	1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Tomatoes	Coburg	2½	After peeler	1	19.3	306	0.51	8.1	0.07	1.1	0.05	0.7	0.67	10.6
			After canning	1	18.2	280	0.46	7.1	0.06	1.0	0.05	0.7	0.66	10.2

Tomatoes	Stokesdale	2	After peeler	2	16.2	275	0.65	10.8	0.07	1.1	0.04	0.7	0.55	9.3
			After canning	2	16.9	294	0.55	9.6	0.06	1.1	0.04	0.7	0.54	9.4
Tomatoes	Coburg	2½	After peeler	1	19.3	306	0.51	8.1	0.07	1.1	0.05	0.7	0.67	10.6
			After canning	1	18.2	280	0.46	7.1	0.06	1.0	0.05	0.7	0.66	10.2
Tomatoes	John Baer	10	After peeler	4	16.5	294	0.55	9.8	0.07	1.2	0.04	0.7	0.60	10.6
			After canning	4	14.6	259	0.46	8.2	0.06	1.1	0.04	0.7	0.59	10.4
Tomatoes	Assorted	2½	After peeler	2	16.8	273	0.60	9.7	0.08	1.2	0.05	0.7	0.70	11.4
			After canning	2	16.1	272	0.51	8.6	0.07	1.1	0.05	0.7	0.70	11.6
Tomato juice	Assorted	46 oz.	After chopper	1	19.0	352	0.58	10.8	0.06	1.1	0.04	0.8	0.64	11.9
			After canning	1	9.9	190	0.42	8.0	0.04	0.9	0.04	0.8	0.62	11.9
Tomato juice	John Baer	10	After chopper	2	18.5	297	0.65	10.4	0.07	1.1	0.05	0.7	0.66	10.8
			After canning	2	14.6	229	0.44	6.9	0.06	0.8	0.04	0.6	0.65	10.2
Tomato juice	John Baer	46 oz.	After chopper	2	19.4	308	0.73	11.5	0.08	1.2	0.06	0.9	0.74	11.7
			After preheater	2	16.7	257	0.63	9.5	0.08	1.2	0.06	0.8	0.68	10.4
			At holding tank	2	15.5	278	0.50	8.9	0.07	1.2	0.05	0.9	0.71	12.8
			After filler	2	14.7	256	0.39	7.0	0.07	1.2	0.05	0.9	0.72	12.5
			After canning	2	14.7	245	0.41	6.9	0.06	1.0	0.05	0.9	0.69	11.5
Tomato juice	John Baer and Stokesdale	46 oz.	After chopper	2	16.6	270	0.57	9.2	0.08	1.2	0.05	0.8	0.75	12.1
			After preheater	2	15.0	237	0.54	8.4	0.07	1.0	0.05	0.8	0.75	11.9
			After finisher	2	14.6	217	0.44	8.0	0.05	1.0	0.04	0.8	0.66	11.7
			After filler	2	13.2	225	0.44	7.4	0.05	0.9	0.04	0.7	0.73	12.8
			After canning	2	13.2	249	0.36	6.8	0.05	1.0	0.04	0.8	0.76	12.4
Tomato juice	John Baer	2 tall	After chopper	2	18.0	282	0.64	9.9	0.07	1.2	0.05	0.8	0.67	10.5
			After preheater	2	16.4	262	0.54	8.6	0.07	1.0	0.05	0.8	0.69	10.8
			At holding tank	2	13.6	251	0.46	8.5	0.06	1.0	0.04	0.8	0.69	12.8
			After filler	2	13.7	262	0.41	7.7	0.06	1.1	0.04	0.8	0.72	13.7
			After canning	2	14.0	224	0.38	6.1	0.05	0.9	0.04	0.7	0.65	10.3
Tomato juice	Stokesdale	2	Before chopper	1	20.5	387	0.66	12.4	0.06	1.2	0.05	0.9	0.54	10.2
			Before finisher	1	17.7	340	0.58	11.2	0.06	1.1	0.05	0.9	0.62	11.2
			After finisher	1	16.1	374	0.51	11.9	0.05	1.1	0.05	1.0	0.60	14.0
			After heater	1	13.5	329	0.36	8.9	0.05	1.1	0.04	1.0	0.60	14.6
			After canning	1	13.2	269	0.37	7.6	0.05	0.9	0.05	0.9	0.52	10.6
Tomato juice	Stokesdale	10	Before chopper	1	20.5	325	0.56	8.9	0.06	1.0	0.05	0.8	0.56	8.9
			Before finisher	1	17.9	271	0.51	7.8	0.06	0.9	0.05	0.8	0.54	8.9
			After finisher	1	16.8	306	0.42	7.6	0.05	0.9	0.05	0.8	0.58	10.5
			After heater	1	12.6	221	0.43	7.5	0.05	0.9	0.05	0.8	0.53	9.3
			After canning	1	12.6	217	0.37	6.4	0.05	0.8	0.04	0.7	0.55	9.5

[1] Uncorrected for the sugar or salt added during canning.

the concentration of the various vitamins as the spears. Blanching and processing appear to have had the same relative effect on vitamin retention in the two types of asparagus. Ascorbic acid and thiamine retention amounted to approximately 50% of the original vitamin content, whereas carotene, riboflavin and niacin retentions were somewhat higher. Vitamin retention was found to be slightly higher in the contents of the no. 2 cans than in the contents of the larger cans. Whether this difference was due to the nature of the product or to processing time cannot be stated with certainty. Since only slight amounts of soluble solids were lost from asparagus during steam blanching, the two bases of expressing vitamin retention yield data for asparagus which are in good agreement.

Beans (whole green)

Eight samplings of whole green beans, packed in four different canneries, were investigated. These consisted of seven samplings of Refugee beans and one sampling of Tendergreen beans. Seven of the eight samplings graded no. 2 (size) while the eighth sampling graded no. 3. All samplings were water blanched for periods varying from 1.5 to 5 minutes at temperatures ranging from 185 to 190°F., depending on the practice of the canner. The blanched beans were packed in no. 2 cans, processed for 20 to 22 minutes at 240°F. and canal cooled.

The beans constituting all eight samplings sustained considerable loss of vitamins during blanching and processing. Both varieties of beans retained approximately 40% of the ascorbic acid (dry weight basis). The beans of the Tendergreen variety retained higher percentages of carotene and thiamine. The latter differences appear to be due to varietal characteristics rather than to slight variations in the canning method. The Tendergreen variety of beans was initially higher in carotene than the Refugee variety.

Beans (cut green)

A total of thirteen samplings of cut green beans were studied. These consisted of eight samplings of the Refugee variety, four of the Tendergreen variety and one sampling of the Bountiful variety. Eleven of the fourteen samplings were graded in respect to size as no. 4 beans. Eight of the samplings represented beans which were eventually canned in no. 2 cans and five of the samplings were from beans consigned to be canned in no. 10 cans. All beans were water blanched for periods ranging from 1.5 to 6 minutes at temperatures ranging from 195 to 200°F., depending on the practice of the different canners. Final processing of all beans was at 240°F. for 20 minutes for no. 2 cans and 35 minutes for the no. 10 cans. The beans canned in no. 2 cans were canal cooled while those canned in no. 10 cans were first cooled in the retort and then in the canal.

As with whole green beans, all samplings of cut green beans sustained appreciable loss in vitamin content during canning. Ascorbic acid retention ranged from 41 to 60%, while carotene and thiamine retention ranged from 81 to 88 and 50 to 71%, respectively, when expressed in terms of dry matter. The Bountiful variety, while considerably higher in initial ascorbic acid content, sustained the greatest loss of this vitamin during canning, while the Tendergreen variety was found to be relatively low in ascorbic acid but exhibited good retention of the vitamin. While the initial carotene content (after snippers) ranged from 2.3 mg per 100 gm dry weight for Grade 3 (size) Refugee beans to 1.2 mg per 100 gm dry weight for no. 5 (size) Refugee beans, the percentage of carotene retained after being canned was essentially the same for the two grades of beans. When computed on the basis of dry weight, approximately 70% of the thiamine was retained by the canned beans. Within the limits of these studies, can size, blanching time and blanching temperature did not appear to have a marked effect on vitamin retention.

Beans (cut wax)

Four samplings of cut wax beans were investigated. The beans constituting all four samplings were no. 4 size and were prepared and canned in two different canneries. The beans were water blanched for periods ranging from 2.75 to 4 minutes at 190°F., packed in no. 2 cans, processed for 20 minutes at 240°F. and canal cooled. Vitamin retention expressed in terms of dry weight ranged from 56 to 57% for ascorbic acid, 59 to 62% for carotene and from 57 to 59% for thiamine. While the initial carotene content of the wax beans was less than one-half that of the green beans, the percentage of carotene retained after canning was about the same for both types of beans.

Beans (bush limas)

Four samplings of bush lima beans, representing two varieties, two commercial grades, two can sizes, and the operation of two canning establishments, were investigated. The beans constituting two of the four samplings were "all-green" limas, while the beans constituting the other two samplings were made up of green and white beans, with the white beans predominating. The beans were water blanched 2 to 4 minutes at 190 to 200°F., depending on the practice of the canner. Final processing was at 245°F. for 25 minutes for the no. 2 cans and 40 minutes for the no. 10 cans. The no. 2 cans were canal cooled, while the no. 10 cans underwent preliminary cooling in the retort and subsequent cooling in the canal.

From the standpoint of vitamin retention, 62 to 74% of the ascorbic acid, 26 to 50% of the thiamine, 72 to 105% of the riboflavin, and 78 to 89% of the niacin originally present in the vined beans was retained by the canned product. Not only did the "all-green" lima beans (Clark's Allgreen) contain a greater initial amount of ascorbic acid than the mixed lima beans (Henderson's Bush), they also retained a greater percentage of the vitamin after being canned. However, this difference may have been partially due to differences in the

maturity of the two varieties of beans when canned. The data suggest that thiamine destruction was considerably greater where the beans were canned in no. 10 cans than where no. 2 cans were used. However, the effect of can size was less significant in the case of riboflavin and niacin retention.

Carrots (diced)

Two samplings of Red Core Chantenay carrots were investigated with respect to change in vitamin content during preparation and canning. Both samplings were taken from the same cannery where the final product was being packed in no. 10 cans. The topped carrots were subjected to a 10-minute water blanch at 212°F., cooled, sorted, diced, filled into cans, processed for 25 minutes at 240°F. and canal cooled.

While there was appreciable loss of ascorbic acid from carrots as the result of blanching, there appeared to have been some reducing substance or substances formed during the subsequent heat treatment which interfered with ascorbic acid measurements. When calculated to the dry weight basis, the data suggest a slightly higher ascorbic acid content for the canned carrots than for the washed unblanched carrots. Since ascorbic acid losses were found to have resulted from blanching, the apparent increase in vitamin content must have been due to the formation of substances which interfere with the method of assay. Carotene retention by carrots during canning was quite satisfactory inasmuch as more than 80% of the original carotene content was found in the canned product. Thiamine and niacin retentions were also satisfactory, approximately 74% of the original content of these vitamins being retained by the finished product.

Cherries

Six samplings of Montmorency cherries, as packed in no. 10 cans by four different canners, were investigated with respect to changes in ascorbic acid and carotene content during the canning operation. After being sorted, washed, pitted and drained, the cherries were placed in cans, boiling water was

added, the cans were exhausted for 20 minutes in a water bath at 155°F. and closed and processed for periods ranging from 16 to 23 minutes at 200 to 212°F., depending on the practice of the canner.

Ascorbic acid retention was remarkably high with all samplings of cherries, an average of 96% of the original vitamin (dry weight basis) being present in the canned product. While carotene retention appears to have been even more satisfactory than ascorbic acid retention, there is some question regarding the proper interpretation of the apparent high carotene values. It appears that either carotene was liberated or made extractable from the fruit tissues by the heat treatment or that other pigments were liberated or formed which subsequently interfered with the carotene assay. The writers believe that the latter suggestion offers the more plausible explanation for the apparent high-carotene value of canned cherries.

Corn (brine-packed, whole kernel)

Seven samplings of brine-packed whole kernel corn were investigated. These samplings represented three different varieties of corn, two different can sizes, and involved the canning operations of four different canning establishments. The corn comprising some of the samplings was steam blanched for periods ranging from 2.0 to 2.5 minutes, while the corn constituting other samplings was processed without blanching. While all samplings were subjected to a certain degree of washing during the canning operation, neither the method of washing nor the duration appeared to be standardized among the different canners. After being placed in cans and sealed, the corns were processed at 240 to 250°F. for periods ranging from 40 to 50 minutes for the no. 2 cans, and for 90 minutes for the no. 10 cans. The corn processed in no. 2 cans was canal cooled while the corn processed in no. 10 cans received preliminary cooling in the retort before being canal cooled.

There appeared to be some loss of ascorbic acid during the canning of corn, especially during the early stages of the canning operation. However, during processing at the higher temperatures, it appeared that reducing substances are formed which interfere with the ascorbic acid estimation. This is indicated by the apparent increase in the ascorbic acid content of corn during the final stages of the canning operation, especially where the vitamin content is calculated to the dry weight basis. Loss of carotene from corn during canning was variable and did not appear to bear any consistent relationship to variety, size of can or specific processing method employed. Frequently the carotene content of canned corn (on dry weight basis) was found to be greater than that of fresh corn direct from the cutter. These high carotene values may have been due to the formation of carotene-like pigments during the canning procedure which complicated the carotene assay or they may have been due to the loss of non-carotene-containing solids from the cut corn during the rinsing or washing, preparatory to the final stages of canning, with the result that the concentration of carotene in the residual solids exceeded the destruction of carotene during the canning operation. The latter hypothesis appears most tenable.

Thiamine retention in canned corn was found to be relatively low inasmuch as it ranged from 25 to 34% of that of the fresh corn direct from the cutters. There was some evidence that the steam-blanched corn retained slightly higher percentages of thiamine than did the corresponding unblanched corn. The loss of nonriboflavin-containing solids during the washing of the corn tended to overshadow any decrease in riboflavin content during the canning operation. Niacin retention in canned corn ranged from 72 to 95%.

Corn (vacuum-packed, whole kernel)

The changes in the vitamin content of four samplings of vacuum-packed corn, representing two commercial varieties, were investigated. The two varieties of corn were packed

unblanched in 307 x 306 cans by two separate canning establishments. Golden Cross corn was processed at 250°F. for 40 minutes while Top Cross corn was processed at 240°F. for 50 minutes. Both products were canal cooled. There appeared to be no marked differences in the amount or the percentage of vitamins retained in the vacuum-packed corn and brine-packed corn.

Peas (sweet)

Ten samplings of peas (sweet), representing three commercial varieties and involving the canning procedures of four different commercial canneries, were investigated. The samplings of peas graded either no. 4 or no. 5 in respect to size and were canned in no. 2 tins. All samplings of peas were water blanched for intervals ranging from 3 to 10 minutes at temperatures ranging from 190 to 210°F., depending on the practice of the canner. The blanched and rinsed peas were transferred to cans, brine was added, the cans were sealed and processed at 240 to 242°F. for 35 to 40 minutes and canal cooled.

Ascorbic acid retention ranged from 47 to 73% while carotene, thiamine, riboflavin and niacin retention ranged from 96 to 124, 39 to 57, 110 to 138, and 52 to 104%, respectively, when calculated on the basis of residual dry matter. When the vitamin content was calculated on this basis, peas which were subjected to prolonged blanching (6 to 10 minutes) were found to have sustained the greatest loss in ascorbic acid and in thiamine and what appeared to be the least loss in carotene, riboflavin and niacin. Apparently the latter situation was due to extensive loss in noncarotene, nonriboflavin and nonniacin solids during blanching. This hypothesis is confirmed since higher carotene, higher riboflavin and higher niacin values were obtained in the rinsed peas after blanching than were found in the fresh peas direct from the viner. Blanching appeared to be the major cause of ascorbic acid loss from peas while heat treatment was largely responsible for loss of thiamine.

Peas (Alaska)

Two samplings of peas (Alaska) were investigated. Both samplings were of the same variety (Super-Alaska), were packed by the same canner, but were packed in different size cans. The peas of both samplings (size grades 1 and 2) were blanched for 6 minutes at 190°F., packed in cans, brine was added, the cans were sealed and processed at 240°F., the no. 2 cans being processed for 40 minutes and the no. 10 cans for 50 minutes. The smaller cans were canal cooled while the larger ones were cooled in the retort before being subjected to canal cooling.

With the exception of carotene, vitamin losses from Alaska peas during canning were found to be considerable. Ascorbic acid, thiamine and niacin retention amounted to approximately 45% (on dry weight basis) while riboflavin retention was approximately 70% and carotene retention about 88%. It is possible that vitamin losses from canned peas were somewhat greater than the above figures indicate and that the actual vitamin loss was partially obscured by loss in water-soluble solids during blanching.

Spinach

Five samplings of spinach (Heavy Pack) were investigated. These samplings, which were being packed in two different sizes of cans, represented the products of two different canneries. One packer employed water blanching while the other blanched with steam. With both methods of blanching, the conveyor type of blancher was used. The blanching time ranged from 1.5 minutes with steam to 4 minutes with water at 195°F. Final processing was at 252°F. for 45 minutes for the no. 2 cans and 60 minutes for the no. 10 cans. All cans of spinach were canal cooled.

Carotene retention by spinach, during canning, was quite satisfactory. However, the retention of the other vitamins was less favorable. Ascorbic acid retention ranged from 58 to 62%, while thiamine, riboflavin and niacin retention ranged

from 23 to 25, 54 to 81, and 49 to 78%, respectively, when calculated on the basis of residual dry weight. The ascorbic acid losses appeared to have occurred primarily during the preprocessing stages of the canning operation. In fact there appeared to be a slight increase in the ascorbic acid content of spinach during processing. However, this apparent increase is believed to be due to the formation of reducing substances during the heat treatment which interfered with the method of assay. Thiamine losses could be attributed principally to the final heat treatment. Riboflavin losses could be attributed, primarily, to the blanching operation while niacin losses were found to occur throughout the canning operation.

Tomatoes

Eleven samplings of tomatoes, representing three commercial varieties, three can sizes and the products of six different canneries, were investigated with respect to changes in their vitamin content during canning. Six of the samplings were composed of graded tomatoes (fancy) while the five remaining samplings (packed in no. 10 cans) were not quality graded. The general practice employed by the majority of the packers was to subject the tomatoes to some type of washing, scalding, sorting and trimming before they were packed into cans. In all samplings, salt was added to the can contents before sealing. The cans of tomatoes from one sampling were subjected to a hot-water exhaust for 23 minutes before the cans were sealed. The cans of tomatoes from six samplings were vacuum-sealed, those from three samplings were closed with a steam-jet sealer, while the cans of tomatoes from the three remaining samplings were sealed with the ordinary closing equipment. All cans of tomatoes were processed at 212°F., the small cans being processed for periods ranging from 46 to 60 minutes, while those in the no. 10 cans were processed 110 to 130 minutes. All cans were subsequently canal cooled.

With the exception of the carotene loss from tomatoes in the unsealed cans during prolonged exhausting (23 minutes)

in a hot water bath, vitamin retention in tomatoes during canning was found to be satisfactory. Ascorbic acid retention ranged from 88 to 100% while carotene, thiamine, riboflavin and niacin retention ranged from 84 to 89, 92 to 97, 91 to 100 and 92 to 100%, respectively, when computed on the dry weight basis.

Tomato juice

Eleven samplings of tomato juice were also investigated. These samplings comprise juices from those varieties of tomatoes most commonly employed in tomato juice manufacture. The eleven samplings of juice were processed by six different canners and were packed in four different sizes of cans. The general packing procedures employed by the different canners were somewhat similar. The tomatoes were washed, scalded, trimmed, chopped, preheated, the juice was extracted, transferred to cans, salt was added, cans were sealed, processed and cooled. Four of the six processors preheated the chopped tomatoes to temperatures ranging from 135 to 156°F. and held them at these temperatures for intervals ranging from 15 seconds to 4 minutes before expressing the juices. The expressed juice was then heated to a temperature ranging from 190 to 196°F. and held at this temperature for periods ranging from 30 seconds to 2 minutes before being transferred to the cans. A fifth processor made it a practice to heat the chopped tomatoes to 180°F. and hold them at this temperature for 2 minutes before expressing the juice. The hot expressed juice was transferred directly to the cans. The sixth processor maintained the chopped tomatoes at 190°F. for 35 minutes before expressing the juice. In this instance, also, the hot juice was transferred directly to the cans. Salt was added to the contents of all cans before the cans were sealed. All samplings of juice were processed at 212°F., ten samplings being processed in vertical retorts, while the eleventh sampling was processed on a conveyor belt through a hot-water bath. Processing time varied from 15 to 25 minutes for the smaller cans to 45 minutes for the no. 10 cans.

Canal cooling was employed with all samplings of tomato juice.

Ascorbic acid retention was found to range from 54 to 92% of that of the chopped tomatoes. The lowest ascorbic acid retention (54%) was found in the juice from the chopped tomatoes which had been subjected to the prolonged preheating (35 minutes at 190°F.) while the highest ascorbic acid retention was in the juice from chopped tomatoes which had previously been preheated to 135°F. for only 15 seconds before the juice was expressed. The expressed juice had been transferred to 46-ounce cans, sealed, processed at 212°F. for 20 minutes and then canal cooled. Carotene retention in tomato juice ranged from 60 to 74%, which was considerably less than the percentage retained by canned tomatoes. Thiamine retention by tomato juice ranged from 75 to 86% which also was somewhat lower than thiamine retention in canned tomatoes. Riboflavin and niacin retention ranged from 89 to 106 and 94 to 107%, respectively.

Since the data obtained in the present investigation showed that many vegetables frequently sustain considerable loss of water-soluble vitamins during blanching, a separate investigation has been instigated for the purpose of ascertaining those conditions of blanching which are conducive to maximum vitamin retention in the blanched product. The results of these latter studies will be published in the near future.

SUMMARY

Studies have been made of the vitamin retention in canned foods as produced under commercial canning practices. The investigation involved nine vegetables and one fruit, and the canning procedures of twenty-seven different canning establishments. A total of eighty-nine different samplings of foods was subjected to vitamin analysis. Samples were taken for vitamin assay as the products entered the canning line and again when the finished product emerged from the cooling canal and, frequently, at intermediate stages along the canning line. These samples were assayed by recognized methods

of assay for two or more of the following vitamins: ascorbic acid, carotene, thiamine, riboflavin and niacin. Samples were also analyzed for dry matter. Vitamin values have been expressed on the basis of the samples as assayed and also on the dry weight basis. The following observations were made:

Ascorbic acid retention was found to be excellent in tomatoes and tomato juice, good in cherries and carrots, fair in asparagus, corn and spinach, and poor in snap beans, lima beans and peas. Carotene retention was excellent in asparagus, cherries, spinach and tomatoes, good in carrots, corn, peas and tomato juice, and fair in snap beans. Thiamine retention was excellent in tomatoes, good in tomato juice, fair in asparagus and carrots, and poor in snap beans, lima beans, corn, peas and spinach. Riboflavin retention was excellent in asparagus, tomatoes and tomato juice, fair in lima beans, corn, peas and spinach, and poor in snap beans. Niacin retention was excellent in asparagus, spinach, tomatoes and tomato juice, fair in carrots and corn, and poor in lima beans and peas.

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DIET OF MOTHER AND HYDROCEPHALUS IN INFANT RATS[1]

L. R. RICHARDSON AND A. G. HOGAN
Department of Agricultural Chemistry, University of Missouri, Columbia

ONE FIGURE

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The literature on the relation between the diet of the mother and abnormalities in the offspring has been summarized briefly by Warkany ('44); therefore the earlier papers will not be cited again at this time. Warkany and Schraffenberger ('44a), and Noback and Kupperman ('44) have described a congenital malformation in the offspring of female rats which received diets that were deficient in riboflavin. Mellanby ('39) observed abnormal development of the teeth and Warkany and Schraffenberger ('44b) observed malformation of the eyes of newborn rats whose mothers received diets deficient in vitamin A. Warkany ('43) reported that skeletal malformation may be present in 45% of the young borne by rats which received a diet that is deficient in vitamin D. Ross et al. ('44) and Cunha et al. ('44) observed congenital malformations, such as syndactylism, talipes, and paralysis agitans in the offspring of sows which received a deficient ration. Hyde ('40) reported that during one 12-month period approximately 18% of the young in an experimental rabbit colony developed hydrocephalus. The epidemic began and ended unexpectedly. The author was quite certain that the abnormality was not inherited, and was of the opinion that it was not due to the diet.

This report describes the occurrence of hydrocephalus in young rats, as a result of inadequate maternal nutrition.

[1] Contribution from the Department of Agricultural Chemistry of the Missouri Agricultural Experiment Station. Journal Series no. 1008.

EXPERIMENTAL

The experimental animals are albino rats and no individuals have been brought in from the outside since the present colony was established 15 years ago. Brother and sister matings have been avoided in recent years. A considerable number of females were provided with Diet A, which contained no crude vitamin carriers to supply unrecognized vitamins. All of the water-soluble vitamins now recognized, except vitamin B_c, were supplied as synthetic compounds. Another group of females received Diet B, prepared by adding to Diet A either 5% of a liver extract fraction or 1% of an eluate of a fuller's earth adsorbate of liver extract. When the females reached maturity they were mated with a male from the stock colony. The composition of Diet A is given in table 1.

TABLE 1

Composition of diet A.

	gm			
Casein, acid-washed and alcohol-extracted	30	Vitamin A	3000	I.U.
Cerelose	52	Vitamin D	425	I.U.
Wood pulp	3	Alpha-tocopherol	2.5	mg
Salts[1]	5	2-Methyl-1, 4-naphthoquinone	2.5	mg
Lard	10	Thiamine hydrochloride	1.0	mg
Choline chloride	0.1	Riboflavin	1.0	mg
Inositol	0.1	Pyridoxine hydrochloride	1.0	mg
P-aminobenzoic acid	0.1	Calcium pantothenate	4.0	mg
		Nicotinic acid	5.0	mg
		Biotin	0.02	mg

[1] The composition of the salt mixture is indicated below:

	gm		gm
$CaCO_3$	267	$FePO_4$, $4H_2O$	20
$CaHPO_4$, $2H_2O$	416	$MnSO_4$, $4H_2O$	25
$MgCO_3$	25	KI	0.6
$MgSO_4$, $7H_2O$	30	$CuSO_4$, $5H_2O$	1.6
NaCl	127	NaF	1.0
KCl	21	$Al_2(SO_4)_3$ K_2SO_4, $24H_2O$	0.24
KH_2PO_4	315	$ZnSO_4$, $7H_2O$	0.5
		$CoCl_2$, $6H_2O$	0.06

In a typical case of hydrocephalus the head is dome shaped and greatly enlarged. The amount of brain tissue is reduced and the brain cavity is filled with serum, which may be either straw-colored or varying shades of pink. Gross examination indicated that the hydrocephalus is internal. In the diagnosis of doubtful cases it is very helpful to observe the transmission of light through the brain cavity. A small box, with an opening 0.25 inches in diameter at one end, was built to enclose a 100 watt light bulb, and the head of the rat is held between the source of light and the observer. A normal brain transmits little or no light, but if a rat has hydrocephalus enough light is transmitted to permit ready detection of advanced cases.

Other symptoms often accompany hydrocephalus, though they do not appear consistently enough to be reliable criteria. The eyes sometime appear to be abnormally small, and in a few cases the eyelids on one side fail to open. Blindness in both eyes has not been observed. If the affected rat survives long enough, muscular incoordination sometimes develops. Hydrocephalus has not been recognized as yet in an animal that was less than 10 days old. It is possible that some of the young on Diet A, which died before they were 10 days old, were affected but death occurred before the symptoms were recognized. Most cases were first observed between the ages of 14 and 20 days, but a few were first discovered at the age of 24 to 28 days. Photographs of typical cases are shown in figure 1, and the observations on the young are summarized in table 2.

A total of 1756 normal young were weaned from the females on Diet A and in addition there were thirty with hydrocephalus. Of these thirty only two survived to the usual weaning age of 28 days. One died 46 days after the condition was first detected. The other was still alive and appeared fairly normal when this manuscript was prepared, 160 days after hydrocephalus was first diagnosed. There were no cases of hydrocephalus in the 1020 young weaned by the females on Diet B, and none in the several thousand young reared by the females in the stock colony. The incidence of hydrocephalus is low

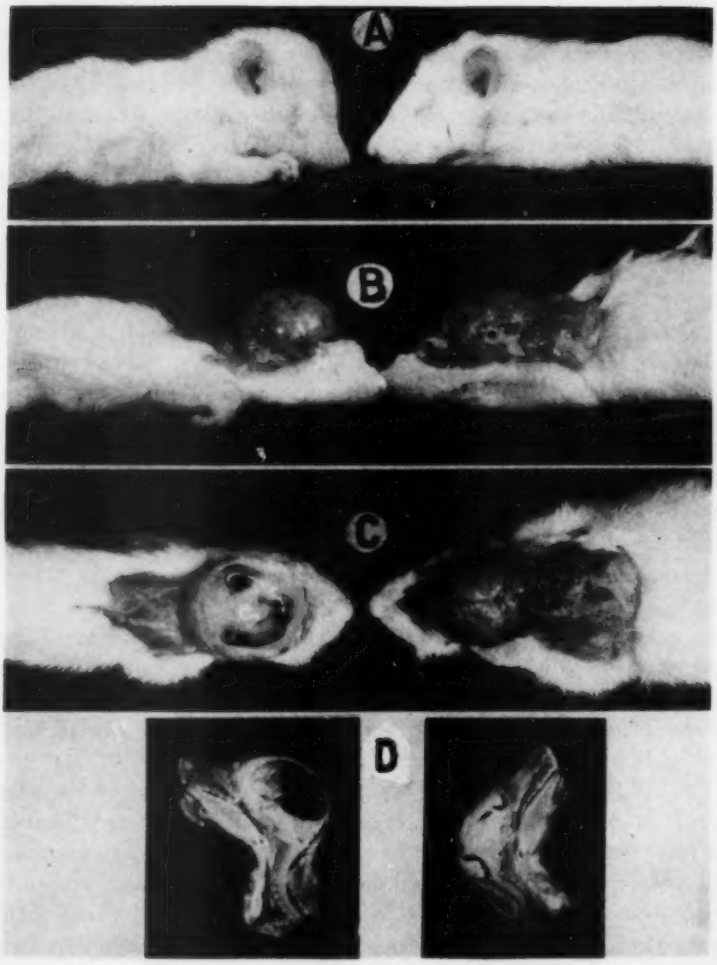

Fig. 1 Hydrocephalic rat on left, normal on the right. A, B, C, photographs of the same rats. D, sagittal section through head of a hydrocephalic and of a normal rat.

but since it has occurred only in young whose mothers received Diet A, it seems reasonable to conclude that the condition is due to a nutritional deficiency. The experimental and control animals were selected at random from the same stock colony and it is assumed that the abnormality has no correlation with genetic constitution. There was one hydrocephalic

TABLE 2

Incidence of hydrocephalus and moisture content of brain of affected animals.

OBSERVATIONS	RATION A	RATION B
Number of females that bore litters	230	54
Number of litters borne	498	175
Number of young weaned	1756	1020
Young with hydrocephalus — Number	30[1]	0[1]
Young with hydrocephalus — Per cent	1.7	0
Number of litters with 1 case of hydrocephalus	18	
Average number of young per litter	4.7	
Number of litters with 2 cases of hydrocephalus	4	
Average number of young per litter	4.6	
Number of litters with 4 cases of hydrocephalus	1	
Number of young in the litter	5.0	
Observations on the brain		
Number of animals	8[2]	12
Average age, days	19	18
Average weight of rats, gm	20.1	30.1
Average weight of fresh brain, gm	1.68	1.06
Average weight of dry brain, gm	0.13	0.18
Per cent of moisture in the brain	92.1	83.8

[1] Statistical analysis by the chi-square method gave a probability of less than 0.1 that the difference was due to chance.

[2] All 8 had hydrocephalus.

young in the litter of a female which received Diet A fortified with 5% of dried brewers blended yeast. One could assume that the yeast does not contain a significant amount of the factor that prevents hydrocephalus. There is also no reason to suppose that the yeast supplied any other factor that was deficient in Diet A, for the percentage of young weaned on

Diet A alone was practically as high as when it was fortified with yeast.

The nutrient which prevents hydrocephalus has not been identified. The liver fractions supplied vitamin B_c, and probably unidentified vitamins in addition. Crystalline vitamin B_c has not been tested by the prophylactic method. Three rats with marked hydrocephalus were each given 500 mg daily of the liver eluate for 4 days, and 2 were each given 100 μg daily of crystalline L. casei factor [2] for 4 days. All five continued to decline and died. Since the three rats which received the liver eluate did not improve it would appear that hydrocephalus can not be cured after it has once developed.

SUMMARY

Female rats received a synthetic diet to which thirteen vitamins had been added. The vitamin mixture did not contain ascorbic acid or vitamin B_c, and the diet contained no unrecognized vitamins unless they were present as contaminants. Nearly 2% of the offspring developed hydrocephalus. Presumably this abnormality was the result of a nutritional deficiency.

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